PHENOTYPING OF HIGH TEMPERATURE SUSCEPTIBILITY IN GARDEN ROSES (*ROSA ×HYBRIDA)*

A Dissertation

by

OCKERT FREDERICK COENRAD GREYVENSTEIN

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Chair of Committee,	David H. Byrne
Co-Chair of Committee,	Terri W. Starman
Committee Members,	Brent H. Pemberton
	Genhua Niu
	Seth C. Murray
Head of Department,	Daniel R. Lineberger

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ABSTRACT

Roses (*Rosa ×hybrida*) have delighted man for nearly 5000 years as ornamentals, food, and medicine. A decline in garden roses in the U.S. has been observed in the past 30 years, which can be attributed in part to the lack of widely adapted cultivars. Adaptation to high temperature stress is viewed as high priority in breeding programs of all major crops. High temperature stress negatively affects garden rose performance and the quality of flowers produced. The work described in this dissertation is focused on quantifying high temperature susceptibility in garden roses to enable breeders selecting for high temperature performance to make better selections. Seasonal change in flower size and plant architecture was investigated on 14 field grown cultivars. Controlled environment experiments were used to establish the developmental stage where flowers were most sensitive to high temperatures. The effectiveness of detached leaf assays as indicators of thermotolerance by way of cell membrane thermostablity (MTS) and chlorophyll fluorescence is reported on. Flower abscission and leaf necrosis of whole plants shocked in a heat chamber were correlated to summer flower productivity. The mean daily maximum temperature for days 8 - 14 (2WkMaxºC) before a flower opens best described the fluctuation in flower dry weight during the growing season. Differences in the rate of change were found among cultivars. Subjecting plants at different stages of development to two week high temperature (36/28 ºC) treatments revealed flowers were most sensitive to high temperatures at the visible bud stage of development. Two week high temperature treatments and high temperature shock (44

ºC, 3 h) both resulted in decreased flower dry weight and increased flower abscission. Initial results favored MTS over chlorophyll fluorescence as indicator of high temperature susceptibility. Further investigation showed no correlation between MTS and summer flower intensity recorded for 18 cultivars. Propensity towards flower abscission and leaf necrosis after a three hour heat shock was negatively correlated ($r = -0.55^{*}$ and $r = -0.64^{**}$) with field ratings of summer flower intensity. Selecting against the propensity towards flower abscission and leaf necrosis under heat stress is suggested as phenotyping tools to select against high temperature susceptibility prior to field establishment of roses.

DEDICATION

This dissertation is dedicated to three great men critical to my being:

Grandfather: Ockert Johannes Albertus Greyvenstein (1919 – 2002)

Grandfather: Hendrik Frederick de Villiers (1921 – 1980)

Father: John Mortimer Greyvenstein (1953 –

"Die hele lewe leef in ons: om onsself te ken – en om selfs intelligent nederig te wees – moet ons al die gestaltes ken wat in ons aanwesig is"

N. P. Van Wyk Louw

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Archeological evidence suggests that man has been cultivating roses as far back as 5000 years in civilizations of China (Krussmann, 1981), western Asia and northern Africa (Shepherd, 1954). During this time roses have served as a food source, medicinal plants and as ornamentals (Gudin, 2000; Shepherd, 1954).

The genus *Rosa* L. comprises of 150 - 200 species (Wissemann and Ritz, 2007) with a range in ploidy (2n – 8n) and are mostly distributed throughout the temperate regions of the Northern Hemisphere (Krussmann, 1981). A biological species concept is not applicable in the case of *Rosa*, as the majority of species appears to be interfertile resulting in viable offspring (Wissemann and Ritz, 2007). Cultivated roses today are the result of numerous interspecific hybridizations of about ten different species (Gudin, 2000). Although genetically similar cultivated roses can be classified based on their horticultural use as: garden roses, pot roses, and cut-roses (Debener and Linde, 2009).

Roses (*Rosa* ×*hybrida*) are part of the top five ornamentals worldwide. Based on numbers from 1995 to 2007 the value of potted roses and cut flowers is estimated to be roughly 24 billion Euros per year (Heinrichs, 2008). The USDA (2010) estimates the wholesale value of potted roses in 2009 at $26 million. The sale of traditional type garden roses such as hybrid teas in the U.S. has been estimated to have declined by nearly 70 percent in the last 30 years. Overall garden rose production in the U.S. declined by about 40 % over the same time period (H.B. Pemberton, 2013, personal

communication). In spite of the decrease in garden rose production, an increase in the sales of a small number of widely adapted non-traditional shrub type roses such as the KnockOut® roses has been observed in the past few years (Hutton, 2012).

The decline in garden rose sales can in part be attributed to the lack of material widely adapted to black spot (*Diplocarpon rosae*) and abiotic stress factors such as high and low temperatures, drought, and salinity (Byrne et al., 2010). High growing temperature of greenhouse produced cut roses has a significant negative effect on flower dry weights (Shin et al., 2001) as well as cut rose flower quality and vase life (Marissen, 2001; Moe, 1975). High temperature shock treatments on cut rose flowers during a sensitive stage of development can reduce the anthocyanin concentration and affect anthocyanin composition in rose flowers (Dela et al., 2003). Production temperature in potted roses can affect plant architecture and can result in more compact plants producing fewer internodes (Clark et al., 1993; Grossi et al., 2004).

Poor adaptation to abiotic stress factors such as high temperatures may be exacerbated by climate changes such as drought accompanied by high temperatures. The area of land globally affected by very dry conditions has increased since the 1970's by approximately 12% (Dai et al., 2004). Modeling of wheat (*Triticum aestivum*) yield based on climatic models over European wheat producing regions predicts high temperature stress during flowering as the largest risk factor for yield loss by 2050 (Semenov and Shewry, 2011). Although the yield of garden roses are not simply measured in product per acre but rather as landscape performance which takes into

account flower productivity, quality of flowers, and overall plant health, we know from literature that rose flowers are sensitive to their growing environment.

1.1 Project goals

This project was initiated to address the observed decline in the sale of garden roses due to the lack of widely adapted material. Adaptation to global climate change is predicted to play an increasing role in crop production. The **long term goal** of this project was to develop garden rose cultivars adapted to high temperature conditions affecting large areas of the southern U.S.

The Texas A&M Rose Breeding Program quantified the yield of garden roses on a 1 - 5 scale. Landscape performance was influenced by the ability of a plant to maintain healthy foliage and the percentage of the rose perpetually covered in blooms. To make breeding progress, an accurate method to quantify any trait of interest is required. Roses are long lived perennials with long generation times that slow progress from selection. It takes roughly three years from pollination until it is possible to evaluate field performance. Establishment of roses in the field is costly and ties up both limited field space and resources to maintain the plants for several seasons.

The specific aims of this dissertation were to: 1) quantify the change in flower quality observed over growing seasons, 2) develop a reliable phenotyping protocol to evaluate and discard the inferior performing material prior to establishment in the field and, 3) rank cultivars in their susceptibility to high temperature stress.

1.1.1 Quantify the seasonal change in flower quality

In addressing the first aim, it was hypothesized that the reduction observed in flower size during high temperature conditions varied significantly among the germplasm in the Texas A&M Rose Breeding program. This hypothesis was supported by field observations, and the fact that a major portion of the germplasm in the Texas A&M Rose Breeding Program was an admixture of two very different breeding programs, donations in early 1990's and 2008. The first was donated by the late Dr. Robert Basye who selected for local adaptation in central Texas (Caldwell, TX). The second set of germplasm was donated by the late Ralph Moore from the Sequoia Nursery in Visalia, CA, where his selection was focused on aesthetic flowering traits. Long term selection under such diverse set of conditions and selection goals are very likely to result in significant genotypic differences with regards to flower productivity.

The first aim was addressed by recording flower size of 14 adapted cultivars planted in beds over a two year period and using weather parameters to model the observed change in flower size (Chapter II).

1.1.2 Development of a phenotyping protocol

Addressing the second aim of this dissertation required the formulation of four more specific hypotheses. The first hypothesis was that there was a critical stage of development where rose flowers are most sensitive to high temperature stress. The first hypothesis was supported by literature suggesting that rose flowers become sensitive to high temperature conditions once the flower has reached the visible bud stage of development (Dela et al., 2003; Shin et al., 2001). The first hypothesis was tested by

subjecting rose plants at different developmental stages to high temperature treatments (Chapter III).

The second hypothesis was that measuring the reduction in chlorophyll fluorescence or electrolyte leakage from cell membranes after a high temperature stress would enable the identification of susceptible material prior to field establishment. The second hypothesis is supported by numerous reports in literature that both chlorophyll fluorescence and electrolyte leakage from cell membranes have been deemed effective in predicting plant performance under high temperature conditions. Chlorophyll fluorescence was used to successfully evaluate high temperature tolerance in an interspecific raspberry (*Rubus*) population (Bravo, 2009). Electrolyte leakage was reported successful in predicting thermo tolerance on field crops such as wheat (*Triticum aestivum* L.) (Ibrahim and Quick, 2001a), twenty different species of vegetables (Kuo et al., 1993), tomato (*Solanum lycopersicum* L.) (Camejo et al., 2005), food legumes (Srinivasan et al., 1996) including cowpeas (*Vigna unguiculata* L.) (Thiaw and Hall, 2004), and ornamental plants such as chrysanthemum (*Dendranthema* x *grandiflora*) (Wang et al., 2008; Yeh and Lin, 2003). The second hypothesis was tested by subjecting whole plants and detached leaves to various high temperature stress conditions and recording chlorophyll fluorescence and electrolyte leakage on leaves of various ages (Chapter IV).

The third hypothesis was that there are genotypic differences within the breeding program with regards to flower abscission and leaf necrosis after a high temperature stress event. The third hypothesis was supported by literature where the differences in

development of necrotic lesions after a high temperature stress has been used to predict thermo tolerance in plants (Levitt, 1980; Sachs, 1864). Tolerance to flower abscission under high temperature stress has been linked to increased yields of beans (*Phaseolus vulgaris* L.) (Monterroso and Wien, 1990) and cowpeas (Hall and Ismail, 1999) during high temperature conditions. The third hypothesis was tested by subjecting rose cultivars to high temperature stress during the sensitive stage of development and recording necrosis and flower abscission following the stress treatment (Chapters III, IV, and V).

The fourth hypothesis was that the genotypic differences detected by way of the above developed screening techniques are predictive of field performance. The fourth hypothesis was tested by evaluating the summer flowering intensity of 18 cultivars over two years and correlating the summer flower productivity to electrolyte leakage, propensity towards flower abscission and leaf necrosis after a high temperature stress event (Chapter V).

CHAPTER II

QUANTIFYING THE FLUCTUATION OF ROSE FLOWER DRY WEIGHT WITH CHANGING ENVIRONMENTAL CONDITIONS

2.1 Synopsis

High temperature stress is a major limiting factor for growing crops and can severely affect rose flower quality and post-harvest longevity. Flower dry weight and number of vegetative nodes to flowering of fourteen adapted garden roses were studied in Texas during 2010 and 2011. A simple linear model based on the average daily maximum temperature (2WkMaxºC) for days 8 - 14 before flowers open divided the 14 cultivars into two groups: cluster one and two with a predicted 4.28 % and 6.45 % decrease in mean flower dry weight for a 1 ºC increase in 2WkMaxºC, respectively. The average mean daily temperature recorded during the measured days was 24.7 ºC, with maximum and minimum daily averages of 34.4 and 2.22 ºC. The number of vegetative nodes to the inflorescence was affected by the growing season but the change and magnitude in the number of vegetative nodes were cultivar dependent. The number of nodes of some cultivars was not affected by the growing season and could be an indicator of high temperature tolerance. Cultivars clustered in two groups based on their change in flower dry weight but could not be categorized according to how the number of nodes changed with the growing season. It might be possible to combine the genetics of stable flower size with desired plant architectures under high temperatures.

2.2 Introduction

Roses are the world's most popular garden plant and cut-flower. Archeological evidence suggests that roses have been cultivated in China from as early as 2737 BC (Krussmann, 1981). It has been estimated that the sale of garden roses in the U.S. has decreased by roughly 30% over the past 20 years (Byrne et al., 2010). According to Hutton (2012) this decrease in sales can in part be attributed to the lack of cultivars adapted to a wide range of climatic conditions. Although an overall decline in garden roses has been observed, an increase in nontraditional shrub roses has been observed in the past few years (Hutton, 2012). This increase in sales in nontraditional shrub roses can be partly attributed to a few widely adapted cultivars such as the Knock Out® series (Hutton, 2012).

One of the major limiting factors for growing crops worldwide, especially in subtropical climates like Texas, is high temperature stress (Wahid et al., 2007). The effect of high temperatures on rose growth and flower development is complex. Higher growing temperature of greenhouse produced cut roses had a negative effect on flower dry weights (Shin et al., 2001) as well as cut rose flower quality and vase life (Marissen, 2001; Moe, 1975). High temperature shock treatments on cut rose flowers during cultivation reduced the anthocyanin concentration and affected anthocyanin composition in seedlings of 'Jaguar' rose flowers, leading to diminished aesthetics (Dela et al., 2003).

The plant architecture of garden roses is in part influenced by the number and internode length of vegetative nodes to the inflorescences. Kawamura et al. (2011) reported that the number of nodes and their lengths were individually controlled,

pointing towards the ability to individually select on internode length and number. Grossi et al. (2004) found when potted miniature roses were produced under summer like conditions, a reduction in the number of vegetative nodes were observed, which corresponded to shorter plants of potted rose cultivar 'Meijikatar' grown during the summer (Clark et al., 1993).

Ambient temperature in combination with humidity is well documented to affect the comfort levels of both humans (Steadman, 1979) and animals (Kelly and Bond, 1971) and have resulted in the formulation of several temperature humidity indices (THIs). Animal scientists, especially in dairy (Bohmanova et al., 2007) and porcine (Zumbach et al., 2008) production have studied the economic and genetic impacts of high temperature stress on animal productivity. Data from local weather stations have been used to model the effects of local weather conditions on milk and fat yield (Ravagnolo et al., 2000). Several THIs have been compared for their effectiveness as indicators of milk production losses and found that weighting of temperature and humidity in such models was dependent on the local climate (Bohmanova et al., 2007). Although the THI accounting for the most variation differed between humid and drier climates, they produced models to predict losses in production per unit increase of the respective THI for each climate.

The effect of environmental factors on greenhouse production of roses has been studied. Computer models such as those developed by Mattson and Lieth (2007) take environmental factors such as temperature, humidity, and light into account to aid growers in planning their rose cut flower crops. The effect of growing temperature on

flower dry weight for the cut rose ‘Kardinal’ was modeled under growth chamber conditions, and the time period prior to visible bud stage did not have a significant effect on flower dry weight (Shin et al., 2001). The temperatures used by Shin et al. (2001) ranged between 15 - 30 ºC and flower dry weight decreased with increasing temperature in a quadratic fashion after the visible bud stage.

Observations from the field indicate that garden roses suffer from loss of flower quality and yield due to high temperatures (D.H. Byrne, personal communication). The effect of environmental conditions on the quality of garden rose flowers has yet to be quantified. Increased sales of a limited number of widely adapted garden roses provided evidence that breeding for these types of roses is likely to help maintain the growth of sales observed in nontraditional shrub type roses. As in major agronomic crops such as maize (Tian et al., 2011), the understanding of genotype by environmental interaction for flower quality and plant architecture traits will be valuable to garden rose breeders for continual improvement of garden roses. The objectives of the chapter were to: 1) propose a mathematical model to quantify the effect of temperature and humidity on the flower dry weight, and 2) to quantify the seasonal change in the number of nodes to the inflorescence of 14 garden rose cultivars commonly grown in hot humid climates in the U.S.

2.3 Materials and methods

2.3.1 Plant material

Established *Rosa* L. plants in 7.8 L pots of 14 cultivars commonly grown in gardens in hot humid climates of the U.S. were obtained from the Antique Rose Emporium, Brenham, TX. The 14 cultivars used were: 'Basye's Blueberry' (BB), 'Belinda's Dream' (BD), 'Caldwell Pink' (CP), 'Carefree Beauty' (CB), 'Earth Song' (ES), 'Folksinger' (F), 'Iceberg' (I), 'Little Buckaroo' (LB), 'Marie Pavie' (MP), 'Old Blush' (OB), 'Quietness' (QN), 'Rise N Shine' (RNS), 'The Fairy (TF), and 'Winter Sunset' (WS). The plants were planted in raised beds on the College Station campus of Texas A&M University during the fall of 2009. Plants were arranged in a randomized complete block fashion with 6 blocks x 2 reps per block x 14 cultivars resulting in 168 individual plants. Plants were irrigated and managed for disease as required.

2.3.2 Flower dry weight

All the plants were pruned in February, May, and September of 2010 and 2011. Pruning was used to synchronize new growth on all cultivars. From here on, the time periods following each pruning until the next pruning will collectively be referred to as the growth period. Flowers were harvested a total of 18 times and harvesting occurred every two to three weeks throughout the growth period. Only flowers that were fully open at the day of harvest were harvested. If more than two fully open flowers were present at the day of harvest two representative flowers were chosen from each plant. If two or less flowers were fully open, all fully open flower(s) were harvested. In 2011 it

was decided that, for cultivars producing small flowers (CP, LB, RNS, and TF), three flowers would be harvested per plant on each harvest day to reduce measurement error. If less than three fully open flowers were present on above mentioned small-flowered cultivars, no flowers were harvested. Flowers were harvested by cutting at the base of the receptacle where the peduncle is attached. Upon harvesting, individual flowers were placed in small envelopes and dried in a drying oven set at 60 ºC to a constant weight. After drying, flower dry weights were recorded to the 0.001g (Mettler AE 50, Mettler-Toledo International Inc., Columbus, OH).

2.3.3 Vegetative growth

Following 2011 the plants were additionally pruned in February of 2012. The number of vegetative nodes to the main inflorescence was counted on the first two flowering shoots for each plant following the May and September 2011, and February 2012 pruning. Counting the vegetative nodes following the above mentioned pruning dates gave the opportunity to follow the vegetative growth after spring (May), summer (September), and winter (February). The number of vegetative nodes to the inflorescence was the number of nodes to the first bract-like leaf before the reproductive bud similar to Kawamura et al. (2011).

2.3.4 Weather data

The weather parameters used in this study were obtained from Weather Undergound, Inc. from the Easterwoord Airport (KCLL) weather station located approximately 2 km from the field location (Weather Underground Inc., 2012). In total,

30 weather variables were calculated from the daily weather report. Nine weather variables for temperature, humidity, and THI were calculated for each day of harvesting. THI was calculated according to the formula developed by Kelly and Bond (1971) where THI = T - (0.55 - 0.0055 x RH) x (T-14.5), where T is temperature in ºC and RH is relative humidity. The nine variables consisted of the daily maximum, daily mean, and daily minimum values for temperature, humidity and THI. The daily maximum, mean, and minimum values averaged over days 1 to 7, days 8 to 14, and days 15 to 21 before harvest resulted in the first 27 weather variables. Additionally the daily minimum humidity in combination with the daily maximum temperature was used in calculating THI for days 1 to 7, days 8 - 14, and days 15 - 21 before harvest, giving a total of 30 weather variables. Principal component analysis (PCA) was performed separately on all the temperature, humidity, and THI data. The first principal components of the above three principal component analyses were saved and used as weather variables, resulting in a total of 33 weather variables for each harvest day.

2.3.5 Statistical analysis

All statistical analyses were performed using JMP software, Version 9.0 SAS Institute Inc., Cary, NC, 1989 - 2010. Modeling flower dry weight occurred in several stages. A $\log_{10}$ transformation (from here on referred to as log) was performed on the flower dry weight to improve normality and fit. The full data set was analyzed by ANOVA as a randomized complete block design. Following ANOVA, the dataset was collapsed over the block effect and an average flower dry weight for each cultivar at each harvest date was calculated. Using the log of the average flower dry weight as the

response variable (y) the following linear model: $y = \mu + cv_i + X + cv*X_i + \varepsilon$, weighted for the number of flowers per cultivar at each harvest date was fit. Where μ is the overall mean, *cv,* is the cultivar with i = 1 - 14, *X* is the weather variable, $cv*X_i$ is the interaction between cultivar i and weather variable, and ε is the residual. The same linear model with each different weather variable was fit, resulting in 33 models. The linear models were compared and ranked using their adjusted R^2 values and corrected Akaike Information Criterion (AICc) (Burnham and Anderson, 2002). Ward's minimum variance method (Sas Institute Inc, 2007) was applied to cluster cultivars based on their slopes for the best models.

A mixed models approach was used to analyze the number of vegetative nodes to the inflorescence for each cultivar at the three growing seasons. Plant replicate nested in block, and the flowering shoot nested within plant and block, were fit as random effects. Cultivar, block, growing season, and their interactions were fit as fixed effects. Variance components were estimated using an all random model, and inferences was made on the mixed models. Without transformation, the residuals were not randomly distributed, a square root transformation in the number of nodes resulted in a better fit. The square root transformation of the data produced similar results as when untransformed data were used. It was decided to make use of untransformed data for the remainder of the analysis. Significant two way interactions were investigated by setting up linear contrasts. To account for multiple testing error, a Bonferroni correction was applied to determine significance of contrasts performed.

2.4 Results and discussion

2.4.1 Weather data

The average daily mean temperature recorded during the growth period for sampling flowers was 24.7 ºC with a maximum and minimum daily average temperature of 34.4 and 2.2 ºC. The average daily mean relative humidity recorded was 61.7 % with maximum and minimum daily average RH of 96 and 28%. The maximum temperature recorded during the growth period was 42.8 ºC and the minimum temperature recorded was -1.7 ºC.

2.4.2 Flower dry weight

A total of 4035 flowers harvested on 18 days were included in the analysis (Fig. 1). The log of the flower dry weight followed a clear cyclical pattern coinciding with the time of the year, with larger flowers during the cooler months and smaller flowers during the hotter months (Fig. 2).

Primary analysis of variance of the log of flower dry weight resulted in a nonsignificant block effect, P value = 0.064, and a highly significant cultivar effect, P value <0.001. Based on the greatest adjusted R^2 and the smallest AICc values for the 33 models, two models were selected that best fit the data. The two models using the first principal component from THI PCA (THI-PC1), and the average mean daily maximum temperature for days 8 - 14 before harvest (2WkMaxºC) weather variables were selected. Both the THI-PC1 and 2WkMaxºC models had highly significant parameter estimates with adjusted R^2 values of 0.95 and AICc values of 145.59 and 184.66, respectively.

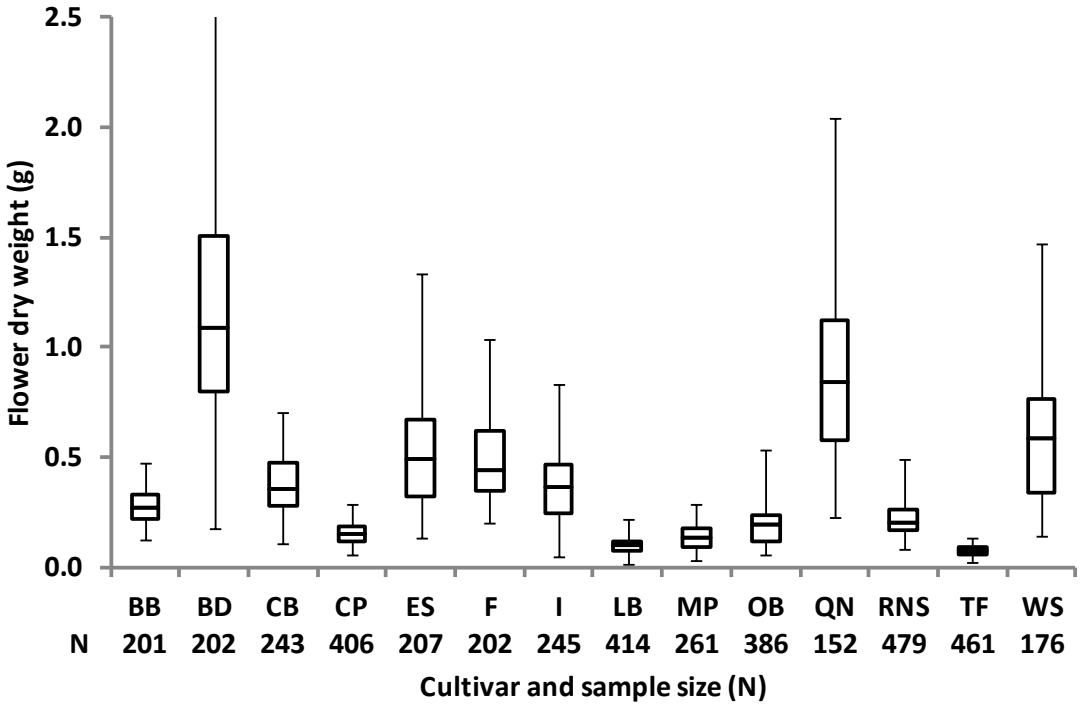

Fig. 1. Box and whisker plot of flower dry weight for the 14 rose cultivars studied in the field with the number of flowers (N) used in analysis below each cultivar.

- 'Basye's Blueberry' (BB), 'Belinda's Dream' (BD), 'Caldwell Pink' (CP), 'Carefree Beauty' (CB), 'Earth Song' (ES), 'Folksinger' (F), 'Iceberg' (I), 'Little Buckaroo' (LB), 'Marie Pavie' (MP), 'Old Blush' (OB), 'Quietness' (QN), 'Rise N Shine' (RNS), 'The Fairy (TF), and 'Winter Sunset' (WS).

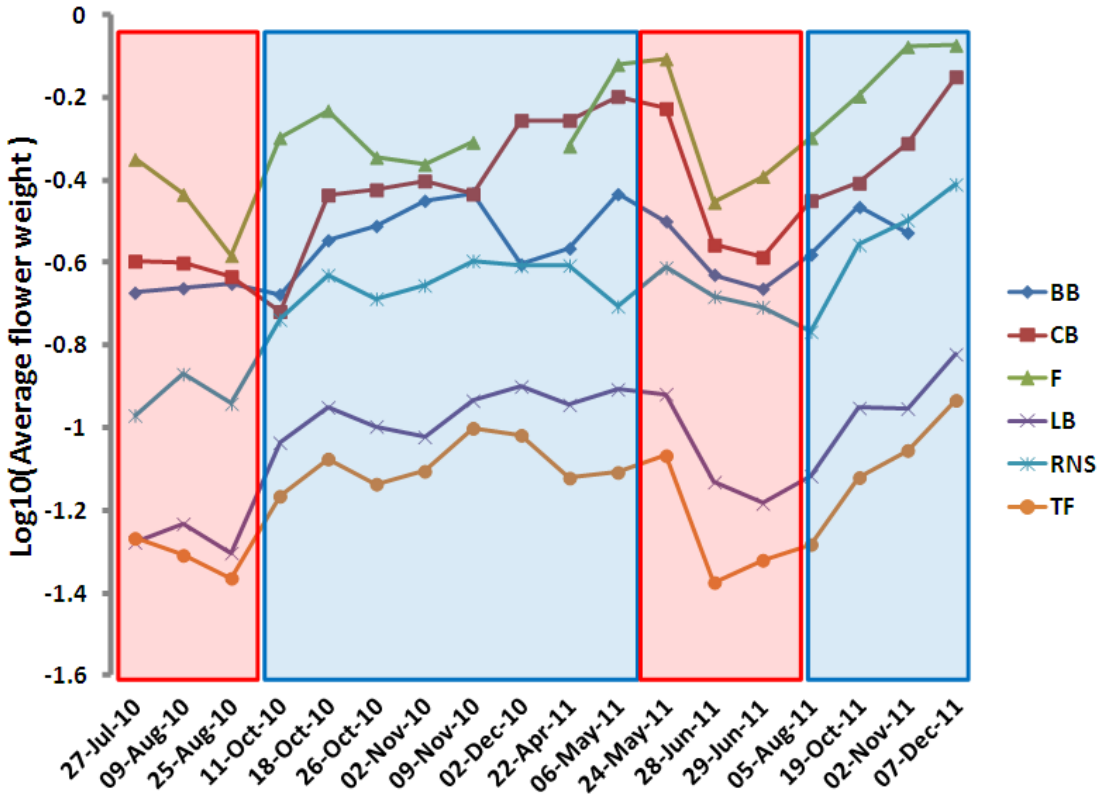

Fig. 2. Log_{10} flower dry weight for a subset of cultivars over the 18 days of harvest.

- Distances between observations are not representative of actual time between observations.
- Red shading indicates warm periods and blue shading cooler periods of growth.
- 'Basye's'Basye's Blueberry' (BB), 'Belinda's Dream' (BD), 'Carefree Beauty' (CB), 'Folksinger' (F), 'Little Buckaroo' (LB), 'Rise N Shine' (RNS), and 'The Fairy (TF).

Models using other weather variables also resulted in significant parameter estimations with high R^2 values. The remaining three principal component models had adjusted R^2 values ranging between 0.93 and 0.94. As for the remaining models using individual weather parameters all temperature and THI models had significant parameter estimates with adjusted R^2 values between 0.90 and 0.93, although their AICc values were greater than the two models selected to best describe the data. The humidity parameter effect was not significant and all models with this weather variable performed poorly in describing the data.

The two best-fit models selected showed a significant cultivar x weather variable interaction, P value < 0.001. The significant interaction indicates differences in slopes for the individual cultivars. Flower dry weight was regressed separately onto the two weather variables, THI-PC1 and 2WkMaxºC, for each cultivar. Ward's minimum variance method clustered the cultivars based on their individual slopes for both THI-PC1 and 2WkMaxºC (Table 1). The clustering resulted in very similar groupings for both weather variables except for QN. Ward's minimum variance clustering method is biased towards producing clusters with roughly the same number of observations (Sas Institute Inc, 2007) and it was the case in this study. Two clusters containing seven cultivars were formed for 2WkMaxºC weather variable, whereas eight cultivars were grouped into the first cluster and six cultivars in the second cluster for the THI-PC1 weather variable.

Table 1. Clustering of cultivars using Ward's method (Sas Institute Inc, 2007) based on the slope of each cultivar when flower dry weight was regressed onto the weather variable.

	2WkMaxºC[z]			THI-PC1[y]		
Cultivar[x]	Adjusted R^2	Slope	Cluster	Adjusted R^2	Slope	Cluster
Basye's Blueberry	0.39	-0.012	1	0.34	-0.018	1
Belinda's Dream	0.71	-0.026	2	0.90	-0.047	2
Carefree Beauty	0.50	-0.022	1	0.49	-0.034	1
Caldwell Pink	0.42	-0.014	1	0.46	-0.025	1
Earth Song	0.62	-0.029	2	0.65	-0.049	2
Folksinger	0.42	-0.020	1	0.40	-0.034	1
Iceberg	0.75	-0.027	2	0.81	-0.045	2
Little Buckaroo	0.67	-0.020	1	0.73	-0.036	1
Marie Pavié	0.81	-0.027	2	0.87	-0.046	2
Old Blush	0.89	-0.033	2	0.94	-0.056	2
Quietness[x]	0.59	-0.027	2	0.61	-0.042	1
Rise N Shine	0.51	-0.019	1	0.61	-0.035	1
The Fairy	0.87	-0.022	1	0.89	-0.038	1
Winter Sunset	0.61	-0.032	2	0.67	-0.055	2

- [z], 2WkMaxºC is the mean average daily maximum temperature for days 14 – 7 before the flower was harvested.
- [y], THI-PC1 is the first principal component after principal component analysis was performed on all the THI parameters.
- [x], Clustered in different groups depending on the weather variable.

Linear models fit for either 2WkMaxºC or THI-PC1 by cluster resulted in models without a cultivar x weather variable interaction (Table 2). The results enabled the formation of two prediction equations per weather variable, each with a different slope for each of the two clusters. Cultivars in cluster 2 had a greater negative slope than cluster 1 cultivars (Fig. 3). Different cultivars within each cluster have different intercepts which corresponds to differences in average flower weights between cultivars (Table 3).

Logarithmic transformations are very useful because logarithms changes multiplicative relations to additive relations and the results are approximately interpretable as percentage changes (Ott and Longnecker, 2001). Interpreting the results as percentage changes makes it possible to compare change in flower dry weight between such a diverse set of rose cultivars. Cultivar BD had the largest flowers with overall mean flower dry weight of 1.22 g whereas TF had the smallest flowers with flower dry weight of 0.07g (Fig. 1). Fair comparison in flower dry weight changes between such a large and small flowering cultivar would not be possible if comparisons were not made based on percentage change. For the 2WkMaxºC model, cluster 1 decreased by a lower percentage than cluster 2 for each unit increase in 2WkMaxºC, and the same for cluster 1 and cluster 2 of the THI-PC1 model. (Table 4).

Table 2. Regression results after fitting the model ($y = \mu + cv_i + X + cv*X_i + \varepsilon$) by clustering for 2WkMaxºC and THI-PC1.

	2WkMaxºC		THI-PC1	
Source of variance	Cluster 1[y]	Cluster 2[z]	Cluster 1[w]	Cluster 2[x]
Cultivar	***	***	***	***
Weather variable	***	***	***	***
Cultivar x weather variable	0.58[N.S.]	0.81[N.S.]	0.41[N.S.]	0.60[N.S.]
Adjusted R^2	0.92	0.94	0.94	0.96

- μ = average flower dry weight, cv_i = cultivar I, X = weather variable, $cv*X_i$ = interaction between cultivar i and weather variable, ε = residual.
- [y, z], Cultivars in cluster 1: BB, CB, CP, F, LB, RNS, TF; cultivars in cluster 2: BD, ES, I, MP, OB, QN, WS.
- [w, x], Cultivars in cluster 1: BB, CB, CP, F, LB, QN, RNS; cultivars in cluster 2: BD, ES, I, MP, OB, WS.
- 'Basye's Blueberry' (BB), 'Belinda's Dream' (BD), 'Caldwell Pink' (CP), 'Carefree Beauty' (CB), 'Earth Song' (ES), 'Folksinger' (F), 'Iceberg' (I), 'Little Buckaroo' (LB), 'Marie Pavie' (MP), 'Old Blush' (OB), 'Quietness' (QN), 'Rise N Shine' (RNS), 'The Fairy (TF), and 'Winter Sunset' (WS).
- [***, NS], Highly significant P value < 0.001, Nonsignificant.

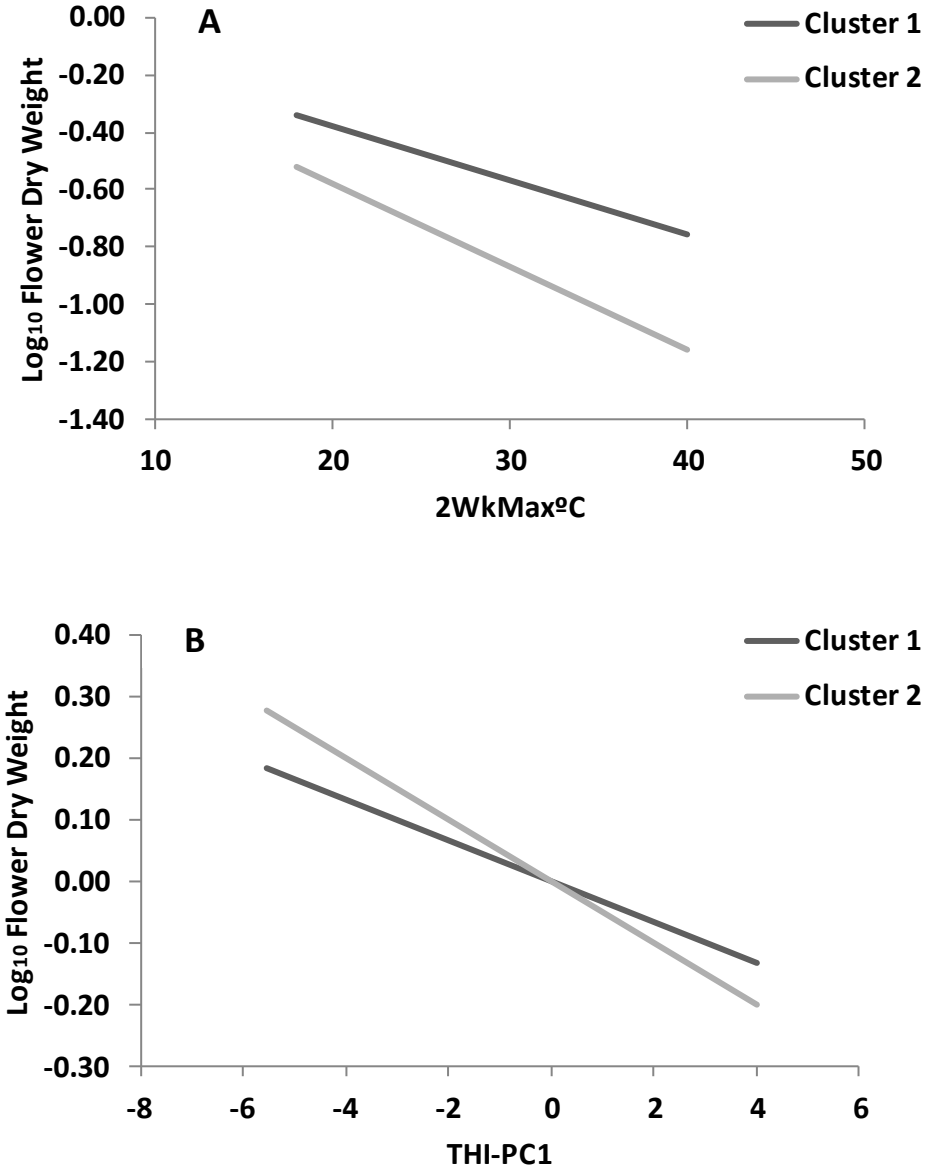

Fig. 3. Prediction equations for different clusters based on 2WkMaxºC (A) and THI-PC1 (B) models with intercepts set at zero.

- 2WkMaxºC cluster 1:BB, CB, CP, F, LB, RNS, TF; cultivars in cluster 2: BD, ES, I, MP, OB, QN, WS.
- THI-PC1 cluster1: BB, CB, CP, F, LB, QN, RNS, TF; cultivars in cluster 2: BD, ES, I, MP, OB, WS.
- Basye's Blueberry' (BB), 'Belinda's Dream' (BD), 'Caldwell Pink' (CP), 'Carefree Beauty' (CB), 'Earth Song' (ES), 'Folksinger' (F), 'Iceberg' (I), 'Little Buckaroo' (LB), 'Marie Pavie' (MP), 'Old Blush' (OB), 'Quietness' (QN), 'Rise N Shine' (RNS), 'The Fairy (TF), and 'Winter Sunset' (WS).

Table 3. Model parameters for prediction equations ($y = B_0 + B_1X$) for change in log_{10} change in flower dry weight.

2WkMaxºC			THI-PC1		
CV	B0[z]	B1[y]	CV	B0	B1
Cluster 1			Cluster 1		
BB[x]	0.071		BB	-0.53	
CB	0.193		CB	-0.412	
CP	-0.221		CP	-0.826	
F	0.315	-0.01901	F	-0.284	-0.03326
LB	-0.412		LB	-1.013	
RNS	-0.062		QN	-0.074	
TF	-0.553		RNS	-0.663	
Cluster 2			TF	-1.154	
BD	1.002		Cluster 2		
ES	0.644		BD	0.083	
I	0.467		ES	-0.268	
MP	0.077	-0.02894	I	-0.452	-0.04996
OB	0.174		MP	-0.842	
QN	0.859		OB	-0.743	
WS	0.672		WS	-0.242	

- [z, y], B_0 = intercept, B_1 = slope for all cultivars within a cluster.
- [x], Basye's Blueberry' (BB), 'Belinda's Dream' (BD), 'Caldwell Pink' (CP), 'Carefree Beauty' (CB), 'Earth Song' (ES), 'Folksinger' (F), 'Iceberg' (I), 'Little Buckaroo' (LB), 'Marie Pavie' (MP), 'Old Blush' (OB), 'Quietness' (QN), 'Rise N Shine' (RNS), 'The Fairy (TF), and 'Winter Sunset' (WS).

Table 4. Percentage changes in flower dry weight per unit increase in weather variables 2WkMaxºC and THI-PC1 for cultivars in each of the two clusters.

	2WkMaxºC[z]		THI-PC1[y]	
	Cluster 1	Cluster 2	Cluster 1	Cluster 2
Slope	-0.019	-0.029	-0.033	-0.049
$10^{(slope)}$	0.957	0.936	0.926	0.891
% Change	-4.283	-6.447	-7.372	-10.867

- [z], 2WkMaxºC is the mean average daily maximum temperature for days 14 – 7 before the flower was harvested.
- [y], THI-PC1 is the first principal component after principal component analysis was performed on all the THI parameters.

Both best-fit models explained the change in rose flower dry weight similarly well. PCA is widely used and accepted as a method to reduce the dimensionality of a set of data, also referred to as dimensionality reduction. Each principal component is produced by using an eigenvector of the correlation matrix with a standardized original variable. The eigenvalues show the variance of each component (Sas Institute Inc, 2007). The first principal component used in the THI-PC1 model explained 85.5 % of the variation in the 12 THI variables used in the PCA. THI-PC1 is comprised of fairly equal weighting of the 12 THI variables ranging from 0.268 to 0.296 for the variables. The four THI variables for days 15 - 21 before harvest received smaller weighting factors (0.268 - 0.288) than did the same four variables for days 1 - 7 (0.293 - 0.296) and 8 - 14 (0.288 - 0.292) before harvest.

When comparing the linear models from the 29 weather variables excluding the models using PCA variables, seven out of the top ten models included weather variables from days 8 - 14 before harvest. Of these top ten models, five were temperature variables and five were THI variables (data not presented). This combined with the smaller weighting factors for days 15 - 21 before harvest in THI-PC1 could be interpreted as evidence that rose flowers are most sensitive to high temperature stress in the second week before harvesting. This agrees with previous work reporting on the effect of high temperature stress on anthocyanin production in 'Jaguar' rose seedlings. A three day 39/18 ºC day/night high temperature treatment in the developmental stage right before flower buds start showing color significantly reduced pigmentation (Dela et al., 2003). Greyvenstein et al. (2012) reported that a two week 36/28 ºC day/night high temperature

treatment on garden roses 'Belinda's Dream' and 'RADrazz' significantly reduced flower dry weights and increased flower abscission. Treatments in earlier developmental stages had a lesser effect on flower dry weight and abscission.

Relative humidity was a poor predictor for flower dry weight. The mean relative humidity (RH) during the growing period was 61.7 % and did not show the same cyclical pattern as daily temperatures. No correlation ($r = 0.001^{NS}$) was found between mean daily temperature and mean daily RH for 2010 - 2011. There was a significant negative correlation ($r = -0.29^{***}$) between daily maximum temperature and daily minimum RH, the significant correlation between daily maximum temperature and minimum RH is consistent with what was reported by Ravagnolo et al. (2000).

An increase in RH can significantly increase the risk of secondary drought injury when plants are exposed to high temperatures (Levitt, 1980). The postharvest quality of 'Baroness' cut roses decreased at higher RH (90 % versus 70% RH) when grown at 22 ºC (Torre and Fjeld, 2001). RH being a poor predictor of flower dry weight was not an indication that RH combined with temperature did not affect the flower dry weight but indicates that the variation in RH was equally distributed among different growth periods. It is well documented that temperature in combination with RH affects the comfort levels for humans (Steadman, 1979) and productivity of livestock (Aguilar et al., 2010; Zumbach et al., 2008). The formula for THI used weights temperature heavy in comparison to humidity. It was thus not surprising that THI variables performed well in explaining the data when the RH models did not perform well.

Interpretation of the THI-PC1 model is not as straightforward as interpretation of the 2WkMaxºC model. Both models adequately explained the data and it is suggested that the 2WkMaxºC model be used for ease of interpretation. A 1 ºC increase in the average daily maximum temperatures for days 8 - 14 before a flower is open can be approximated as a 6.45 % decrease in the average flower dry weight for cultivars in cluster 2 when using the 2WkMaxºC model, similarly is a 4.28 % reduction in flower dry weight predicted for cultivars in cluster 1 (Table 4). It is important to note that the model presented here is only valid within the temperature range experienced during the experiment. The 2WkMaxºC recorded over the 18 sample dates had a maximum of 38.9 ºC, a mean of 31.8 ºC, and a minimum of 18.5 ºC.

By taking the log of the predicted quadratic change in flower dry weight for cut rose 'Kardinal' presented by Shin et al. (2001) at temperatures ranging between 13 and 34 ºC, the following linear model: Log(flower weight) = 1.175 – 0.0462 (growing temperature ºC) had a 0.99 coefficient of determination. A slope of – 0.0462 translates into a 10.1 % ((1-(10^{slope})*100)) change in flower dry weight for a one unit increase in growing temperature, after the flower has reached the visible bud stage. When the 2WkMaxºC model was individually fit for each cultivar, OB had the greatest slope (Table 1) translating into a 7.3 % decrease in flower dry weight per 1 ºC increase in 2WkMaxºC. Considering that 'Kardinal' is a large flowering cut rose and not an adapted garden cultivar, a 10.1 % change in flower dry weight per 1 ºC increase in growing temperature is in line with what was observed in this study.

Possible contributions to decreased flower size under high temperatures could be a combination of reduced photosynthesis and leaves exporting fewer carbohydrates to the flowers under high temperatures. Carbohydrate export and photosynthesis in rose leaves are reduced by 80 and 40 % respectively in leaves at 40 ºC compared to leaves at 25 ºC (Jiao and Grodzinski, 1998). A reduction in petal number and size also affects the flower dry weight (Shin et al., 2001). A reduction in petal number and size was observed during the warm season but was not recorded.

The differences in expected rate of decrease in flower dry weight between the two cultivar clusters provide insight on the high temperature tolerance of flower quality. Looking at the grouping for cultivars it is clear that based on the mean flower dry weight the majority of the large flowering cultivars are grouped into cluster 2 and the majority of the smaller flowering cultivars are grouped into cluster 1, which are more heat tolerant. It is however interesting that the larger flowering cultivar F is placed in cluster 1 and that smaller flowering cultivars such as MP and OB are grouped in cluster 2. The grouping of cultivars is not solely dependent on the flower size and the grouping could be used as an indicator of high temperature tolerance. Four (BD, CB, CP, and TF) of the 14 cultivars used in this study have been labeled as Earth-Kind®. Roses are designated as Earth-Kind® after demonstrating outstanding landscape performance in a wide range of soil types and conditions, including tolerance to pests (Collart et al., 2010). All Earth-Kind® designated cultivars with the exception of BD were grouped in cluster 1. Based on change of flower dry weight under high temperature conditions cultivars BB, F, LB, and RNS performed equally well as Earth-Kind® cultivars, this information could prove

valuable in cultivar selection for hot and humid conditions such as those experienced in southeast Texas.

2.4.3 Vegetative growth

All fixed effects with the exception of block were found to be significant (Table 5). The main effects of cultivar and season accounted for the majority of the variance explaining 62 and 19% of the variance, respectively. The block x season interaction and the cultivar x season interaction accounted for 5% and 8% of the variance, respectively. The significant block x season interaction was due to plants in blocks 1 and 2 having greater number of nodes. Only a small amount of variation is explained by this interaction and at this point no meaningful interpretation is apparent. It is possible that not all blocks responded equally to pruning resulting in the significant block x season interaction.

Of greater interest was the cultivar x season interaction and the cultivar effect. In total, 42 linear contrasts between dates within cultivars were performed to further investigate the cultivar x season interaction. All pairwise comparisons between the number of nodes among cultivars resulted in significant cultivar differences (Table 6).

Table 5. Mixed model F-ratio results and predicted variance component of random effects for the number of vegetative nodes to the inflorescence following the spring, summer, and winter growth periods for 14 cultivars of garden roses.

Source of variance	F value	Variance component	Lower 95%	Upper 95 %
Block	2.01^{NS}			
[z]Plant [y][block]	2.62^{NS}	0.057	-0.057	0.055
[z]Shoot [block,plant]	0.84^{NS}	-0.011	-0.068	0.046
Cultivar	102.37^{***}			
Season	32.02^{***}			
Cultivar x season	12.86^{***}			
Block x season	8.67^{***}			

- [z], Included in the model as random effect.
- [y], [ ], Denotes nesting structure.
- [***, NS], Highly significant P value < 0.001, Nonsignificant.

Table 6. Overall mean and means for the number of vegetative nodes to the inflorescence for 14 garden rose cultivars following pruning after, spring (May 2011), summer (September 2011), and winter (February 2012) growing seasons.

Cultivar	Spring 2011	Summer 2011	Winter 2012	Overall Mean
Basye's Blueberry	13.79 ± 0.45b[y]	19.74 ± 0.41a	20.38 ± 0.38a	17.97 ± 0.25a[z]
Belinda's Dream	12.68 ± 0.41a	12.49 ± 0.41a	13.51 ± 0.40a	12.89 ± 0.24de
Carefree Beauty	10.29 ± 0.37a	10.75 ± 0.37a	11.58 ± 0.37a	10.87 ± 0.22g
Caldwell Pink	13.79 ± 0.37b	15.33 ± 0.37ab	15.46 ± 0.37a	14.86 ± 0.22bc
Earth Song	11.00 ± 0.42b	12.19 ± 0.38ab	13.15 ± 0.38a	12.11 ± 0.23ef
Folksinger	12.03 ± 0.38b	14.10 ± 0.41a	15.41 ± 0.38a	13.85 ± 0.23cd
Iceberg	7.20 ± 0.40b	8.95 ± 0.43ab	10.20 ± 0.40a	8.87 ± 0.24h
Little Buckaroo	14.4 ± 0.38a	13.31 ± 0.38a	10.96 ± 0.38b	12.89 ± 0.23de
Marie Pavié	8.73 ± 0.40b	9.34 ± 0.41ab	11.00 ± 0.40a	9.69 ± 0.24h
Old Blush	11.79 ± 0.37a	12.13 ± 0.37a	11.15 ± 0.38a	11.69 ± 0.22fg
Quietness	14.63 ± 0.37b	13.99 ± 0.38b	16.96 ± 0.37a	15.19 ± 0.22b
Rise N Shine	13.13 ± 0.37a	11.33 ± 0.37b	11.96 ± 0.37ab	12.14 ± 0.22ef
The Fairy	11.65 ± 0.48a	13.2 ± 0.43a	11.60 ± 0.42a	12.15 ± 0.26ef
Winter Sunset	13.53 ± 0.39a	11.28 ± 0.40b	11.85 ± 0.40ab	12.22 ± 0.24ef

- [y], Seasons not connected by the same letter are significantly different at $0.05 \div 42 = 0.0012$, contrasts were performed between growing season within each cultivar.
- [z], Cultivars within overall mean not connected by the same letter are significantly different at $\alpha = 0.05$, with Tukey's adjustment.

Increased temperatures during axillary bud formation resulted in a decreased number of nodes on rose cultivars ‘Mortea’ and ‘Sweet Promise’ (Marcelis-Van Acker, 1995). Axillary buds formed at 25 ºC resulted in shoots with fewer nodes than those from buds formed at 17 ºC for both cultivars. Marcelis-Van Acker (1995) concluded that elevated temperatures experienced by the parent shoot will result in reduced leaves in the following growth. Greyvenstein et al. (2012) reported no change in the number of nodes to the flower for garden roses ‘Belinda’s Dream’, ‘RADrazz’, and ‘Sea Foam’ when subjected to two week periods of high temperatures during differing stages of development. This is in agreement with Marcelis-Van Acker (1995) as all the axillary buds were formed under the same conditions.

All plants were pruned on the same date and the axillary buds were presumably formed under the same conditions. The average daily maximum temperature during May (spring) and September (summer) 2011 and February (winter) of 2012 were 31.7, 39.9 and 18.8 ºC. Given the conditions prior to pruning, the smallest number of nodes would be expected after the summer pruning followed by growth after spring with the greatest number of nodes following the winter pruning. Our results were not as expected. The overall mean number of nodes following the three pruning dates was significantly different from each other. Growth following the winter had the greatest number of nodes (13.23), followed by growth after the summer (12.72) and then growth after spring (12.05). Whether a difference of less than one node is biologically significant is debatable. A confounding effect could be plant age, as the plants were older following the winter, and that older plants are more robust and could possibly support more

growth. This is unlikely to be the case as the plants were established in 2009 and the starting material was established roses in 7.8 L pots so there should be little differences in plant age.

Cultivars CP, ES, I, and MP had fewer nodes following the spring and more following the winter, with the number of nodes following the summer not being different from either of the other two periods. This indicates that these cultivars including QN have reduced number of nodes under warmer (following spring and summer) growing conditions. Cultivars: BD, CB, OB, and TF had the same number of nodes in all three growing seasons and the number of nodes to inflorescence was not affected by the growing conditions. Cultivars not having their growth affected by increased growing temperatures could be of use in breeding programs where high temperature tolerance is concerned.

Cultivars RNS and WS had the least number of nodes following the summer pruning and did not show differences between the other two growing periods. These two cultivars are more affected by the high temperature than the cultivars that showed no change but not as susceptible as CP, ES, F, I, MP, and QN. At this point it is difficult to interpret the results of cultivars LB and BB. It is possible that LB was severely stressed during 2011 and did not fully recover during the winter leading into the spring of 2012. There was no difference in number of nodes for BB following the summer and winter growth periods although the number of nodes was reduced following the spring pruning. Any explanation on the behavior of BB would be speculation at this points and needs to be further investigated.

Overall the cultivars behaved differently with regards to the number of vegetative nodes to the inflorescence and a range of different numbers of vegetative nodes to the inflorescence was represented in the material (Table 6). The effect of number of vegetative nodes on plant architecture and time to repeat bloom is not well described in literature and no data was collected on the first day to bloom after pruning during this experiment. The number of vegetative nodes and the average internode length are individually controlled traits (Kawamura et al., 2011). Some cultivars such as CP and RNS produce on average a greater number of vegetative nodes than OB and ES but are much more compact, owing their compactness to shorter internode lengths.

2.5 Conclusions

Flower dry weight of garden roses showed a marked decrease with increasing temperatures. The decrease in flower size can be approximated as a 4.28 - 6.45 percentage decrease for every 1 ºC increase in the average maximum temperature for days 8 - 14 before the flowers are fully opened. The rate of change in flower size could be an indicator of high temperature tolerance, even between adapted cultivars such as those used in this study.

The number of vegetative nodes to the inflorescence was affected by growing temperatures, but the effect and magnitude was cultivar dependent. The predicted changes in flower size due to changes in growing temperatures appear to be separate from the change in vegetative nodes to the inflorescence. Cultivars clustered in two groups based on their change in flower dry weight but could not be categorized according to how the number of nodes changed with the growing season.

This is the first report quantifying the change in flower size and number of vegetative nodes on such a diverse set of garden roses. These results and techniques could aid breeders in better selecting heat tolerant material to provide the landscape market with quality plants.

Rose breeders will gain from the knowledge of how growing conditions affect plant architecture combined with the knowledge on the effect of growing conditions on flower quality traits of planned breeding crosses. It might be possible to combine the genetics of stable flower size with desired plant architectures under high temperatures, as these traits appear to be individually controlled and this would be a desirable target for future research and breeding.

CHAPTER III

EFFECT OF TWO WEEK HIGH TEMPERATURE TREATMENT ON FLOWER QUALITY AND ABSCISSION OF *ROSA* ×*HYBRIDA* 'BELINDA'S DREAM' AND 'RADRAZZ' UNDER CONTROLLED GROWING ENVIRONMENTS

3.1 Synopsis

The decline in sales of garden roses can, in part, be attributed to the lack of well adapted cultivars. Successful selection for any trait requires an accurate phenotyping protocol. Apart from field screening, a protocol for phenotyping high temperature tolerance in garden roses is yet to be established. An experiment was conducted to determine the stage of development when flowers were most sensitive to high temperature stress. Rooted liners of *Rosa* ×*hybrida* 'Belinda's Dream (BD) and the Knock Out® rose 'RADrazz' (KO) were planted in 0.72 L pots in a peat:perlite media and grown in a greenhouse. Well established plants were pruned retaining several nodes with leaves on two main shoots and treatments commenced. The experiment was conducted in two growth chambers held at either 24/17 ºC (control) or 36/28 ºC (stress) day/night temperatures. Six time and duration temperature treatments included eight weeks of continuous control conditions, eight weeks of continuous stress conditions, and four sequential two-week high temperature shock treatments whereby plants were exposed to stress conditions during weeks 1-2, weeks 3-4, weeks 5-6, or weeks 7-8 with

the balance of the 8 weeks under control conditions. Flower data were collected on the first flowering shoot of each plant. Flower abscission was determined on a whole plant level. Continuously stressed plants flowered in the least amount of days, but did not differ from the continuous control treated plants based on nonlinear thermal unit accumulation until flowering. Both cultivars had a 70% reduction in flower size under continuous stress conditions. Flowers were most sensitive to high temperature stress at the visible bud stage, which corresponds to weeks 5-6 and weeks 7-8 for BD and weeks 3-4 and weeks 5-6 for KO respectively. KO was more tolerant to flower abscission than BD when treated at the visible bud stage but no difference in flower size reduction between BD and KO was found. The number of vegetative nodes to the flower was not affected by treatment and differed between the cultivars.

3.2 Introduction

A decrease in the sale of garden roses in the U.S. has been observed in the past 20 years (Byrne et al., 2010), which can, in part, be attributed to the lack of well adapted cultivars (Hutton, 2012). One of the major limiting factors for growing crops worldwide, especially in sub-tropical climates like Texas, is high temperature stress which can cause irreversible damage to plant growth and development. An approach to manage this issue is the development of plants with high temperature tolerance. Tolerant plants would be able to produce an economically viable yield under high temperature conditions (Wahid et al., 2007).

The effect of high temperature stress on rose growth and development is complex. Excessively high or low growing temperatures negatively impact the longevity

and quality of cut roses (Marissen, 2001; Moe, 1975). Several models describing rose shoot growth and development using ambient temperature and thermal unit accumulation have been developed for greenhouse rose production (Mattson and Lieth, 2007; Pasian and Lieth, 1994; Steininger et al., 2002). Growers can use software tools to model and schedule rose crops. An upper threshold where development is impaired is commonly included when calculating growing degree days for agronomic crops such as maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Mcmaster and Wilhelm, 1997). Although not included by Pasian and Lieth (1994), such a threshold for potted miniature rose 'Candy Sunblaze' was presented by Steininger et al. (2002) as 25.6 ºC for development from bud break until flowers open.

Evidence from literature suggests that rose flower size and quality are most sensitive to high temperature stress at or after the visible bud stage of development. Growing temperature prior to the visible bud stage did not affect the size of 'Kardinal' roses. Flower size quadratically decreased with increasing growing temperature after the visible bud stage (Shin et al., 2001). Loss of rose flower quality by way of anthocyanin reduction was most severe when 'Jaguar' seedlings were subjected to a three day 39/18 ºC day/night high temperature stress at the stage right before flower buds started showing color (Dela et al., 2003).

Rose plant architecture is influenced by the growing temperature. Grossi et al. (2004) reported a reduction in the number of vegetative nodes when potted miniature roses (cultivar 'Meijikatar') were produced under summer like conditions when compared to plants grown under winter like conditions, which corresponds to shorter

plants (Clark et al., 1993). Kawamura et al. (2011) reported a strong positive correlation (r = 0.66) between the number of vegetative nodes and the days to flower on a diploid rose population segregating for perpetual flowering.

Field observations show that garden roses suffer from loss of flower quality and yield due to high temperatures. These observations also point to a wide range of variation in garden rose accessions with regards to performance under high temperature conditions. To our knowledge, this variation has yet to be quantified. The first step to quantify and ultimately breed for any trait of interest is an accurate and repeatable method of phenotyping.

The objectives of this study were to identify the stage of shoot development where flower size and abscission are most sensitive to high temperature stress, and how the number of vegetative nodes and time to flowering is affected by high temperature stress on two garden rose cultivars.

3.3 Materials and methods

3.3.1 Plant material

Rooted liners of *Rosa* ×*hybrida* cultivars 'Belinda's Dream' (BD) and 'RADrazz' (KO) were planted in 0.72 L pots in a peat : perlite (LC-1) (SunGro Horticulture, Bellevue, WA), and grown in greenhouse conditions at the Texas A&M AgriLife Research and Extension Center in Overton, TX. The plants were fertilized with a 200 $mg \cdot L^{-1}$, 15N – 5.4P – 14.1K liquid fertilizer. After the plants were well established, the plants were pruned similar to what was described by Grossi. et al.

(2004), with the modification of leaving two main shoots for buds to develop from instead of just one and more than 3 or 4 leaves on each remaining shoot. Pruning was performed to synchronize flowering.

3.3.2 Experimental setup

A factorial design was used with two cultivars x six treatments x seven replications. The plants were arranged in a randomized complete block fashion. The experiment was repeated at two locations. The whole experiment was conducted in growth chambers (Conviron model E-15, Winnipeg, Manitoba, Canada). The plants were subjected to six time and duration temperature treatments which included eight weeks of continuous control conditions, eight weeks of continuous stress conditions, and four sequential two-week high temperature shock treatments whereby plants were exposed to stress conditions during weeks 1-2, weeks 3-4, weeks 5-6, or weeks 7-8 with the balance of the 8 weeks under control conditions. The experiment was terminated on day 70. The experiment was conducted at the Texas A&M AgriLife Research and Extension Center at Overton and repeated at the Borlaug Center on the College Station campus of Texas A&M University.

A 14 hour photoperiod was maintained at a photosynthetic photon flux density (PPFD) of 570 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LI-191 Line Quantum Sensor, LI-COR®, Lincoln, NE). The day/night temperatures for the control and high temperature conditions were maintained at 24/17 ºC and 36/28 ºC with 70% relative humidity. During the course of the experiment the plants were watered as necessary with a 200 $mg \cdot L^{-1}$, 15N – 5.4P – 14.1K liquid fertilizer.

Flower developmental stages were not quantified in any way although a note was made during which the flower buds became macroscopically visible without touching any leaf, from here on referred to as the visible bud stage. Data was recorded for each plant on the day the first flower fully opened. Days to flower, the number of vegetative nodes to flower, and flower dry weight was recorded for the first flowering shoot on each plant. Flower abscission was evaluated on a whole plant level and scored on a binomial scale; abscission was scored if two or more shoots on a plant had abscised flowers. Plants that did not flower by the end of the experiment were scored as abscised. If plants flowered before or within three days of a high temperature treatment, data were collected and the plants were considered as control treated plants during analysis. Proportionate flower size was calculated by taking the mean flower dry weights of plants grown continuously under control conditions and expressing each observation as a proportion of the mean flower dry weight of the control observations for each cultivar at each location.

The amount of thermal units (TU), in hours, accumulated until flowering was calculated for each plant based on two equations:

$$TU1 = \sum_{j=1}^{r} max\left[\left(T_j - T_{b1}\right), 0\right]\Delta t_j \quad [1]$$

$$TU2 = \sum_{j=1}^{r} max\left[T_i - T_{b2} + k\left(T_j - T_i\right), 0\right]\Delta t_j \quad [2]$$

where r = the number of days to flower, T_j = is the average air temperature (ºC) over a period j, Δt_j is the length of time period j (24 hours) and T_b is the base temperature

(Thornley and Johnson, 1990). The base temperature (T_{b1}) used in Eq. [1] was reported by Pasian and Lieth (1994) as 5.2 ºC for 'Cara Mia' hybrid tea roses. In Eq. [2] T_i refers to the temperature where TUs are not linearly accumulated (Steininger et al., 2002) and was reported by Steininger et al. (2002) to be 25.6 ºC for miniature rose 'Candy Sunblaze', T_{b2} was reported as 9.5 ºC for 'Candy Sunblaze'. The term k is the ratio of the slope for the regression line at $T_j > T_i$ and $T_j < T_i$, and was reported as 0.47 for 'Candy Sunblaze' by (Steininger et al., 2002). The TUs accumulated were calculated by summing the TUs until flowering.

3.3.3 Statistical analysis

All statistical analysis was performed using JMP software, Version 9.0, SAS Institute Inc., Cary, NC, 1989 - 2010. Analysis of variance was performed by fitting a Standard Least Squares model, where the replication effect was considered as a random effect. Tests of equal variance between locations were performed prior to performing analyses combined over locations. Differences between means were tested using Tukey's honestly significant difference test. Significant interaction effects were further investigated by way of linear contrasts. Nominal regression was used to analyze flower abscission data and differences between treatments were evaluated based on odds ratios.

3.4 Results

3.4.1 Flower dry weight

Cultivar BD has larger flowers than KO. For better comparison between the cultivars, flower dry weight was analyzed as the proportionate flower weight. Based on

Levene's equal variance test there was no evidence of unequal variance (P value = 0.389), and data from both locations were combined. KO did not have any plants flowering in treatment wk 7-8, and the cultivar x treatment term was not considered. Thus, each cultivar was analyzed separately over both locations. Only the main effects of location and treatment were significant for both cultivars (Table 7). Plants of both cultivars, BD (23%) and KO (12%), produced larger flowers in the Overton as compared to the College Station trial experiment.

Plants grown in continuous high temperature conditions produced the smallest flowers, whereas plants grown in continuous control conditions resulted in the largest flowers in both BD and KO (Table 8). Flower size of both BD and KO produced under continuous high temperatures were reduced to approximately 30 % of the size of flowers grown under continuous control conditions. The greatest effect on flower size on both cultivars was during the visible bud stage. As the cultivars flowered differently, this corresponded to stress during wk 5-6 and wk 7-8 for BD (46% reduction in size) and wk 3-4 and wk 5-6 for KO (38% reduction in size). Pooling the proportionate flower weights of BD for wk 5-6 and wk 7-8, and for KO during wk 3-4 and wk 5-6 resulted in the proportionate flower size not being different between cultivars.

Table 7. ANOVA F-ratio results for different traits analyzed separately for 'Belinda's Dream' (BD) and 'RADrazz' (KO) after plants were subjected to high temperature stress during different stages of development.

Source of variance	Degrees of freedom		P flower weight[z]		Days to flower		TU1[y]		TU2	
	BD	KO	BD	KO	BD	KO	BD	KO	BD	KO
Location	1	1	12.91^{x*}	4.80^{*}	10.10^{*}	19.02^{***}	12.98^{**}	15.47^{**}	11.31^{*}	17.92^{**}
Treatment	5	4	18.43^{***}	46.71^{***}	9.00^{***}	17.11^{***}	9.02^{***}	50.00^{***}	3.49^{*}	7.29^{***}
Location x treatment	5	4	2.64^{NS}	2.45^{NS}	0.97^{NS}	5.46^{***}	1.04^{NS}	4.59^{*}	0.98^{NS}	5.19^{**}

- Seven replications per treatment were included at each location.
- [z], Proportionate flower weight.
- [y], TU1 and TU2 refers to thermal unit accumulated in hours calculated based on following equations: $TU1 = \sum_{j=1}^{r} max\left[\left(T_j - T_{b1}\right), 0\right]\Delta t_j$ and $TU2 = \sum_{j=1}^{r} max\left[T_i - T_{b2} + k\left(T_j - T_i\right), 0\right]\Delta t_j$. Where r = number of days to flower, T_j is the average air temperature (ºC) over period j, Δt_j is the length of time period j (24 h), T_{b1} and T_{b2} is the base temperature (5.2 and 9.5 ºC), T_i is the temperature where thermal units are not linearly accumulated (25.6 ºC), and k is the ratio of the slope for the regression line at $T_j > T_i$ and $T_j < T_i$ (0.47) (Pasian and Lieth, 1994; Steininger et al., 2002).
- [x], NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

Table 8. Proportionate flower size (± 1 standard error of the mean) over different high temperature stress treatments for 'Belinda's Dream' (BD) and 'RADrazz' (KO).

Treatment	BD		KO	
Control	0.987 ± 0.067	a[z]	1.002 ± 0.034	a
Week 1-2	0.893 ± 0.059	a	0.988 ± 0.047	a
Week 3-4	0.861 ± 0.07	a	0.613 ± 0.047	b
Week 5-6	0.536 ± 0.117	b	0.623 ± 0.074	b
Week 7-8	0.543 ± 0.103	b	-	-
Stress	0.333 ± 0.052	b	0.323 ± 0.04	c

- [z], Levels not connected by the same letters are significantly different at $\alpha \leq 0.05$, with Tukey's adjustment.

3.4.2 Flower abscission

Separate Chi-square analyses for location, cultivar, and treatment indicated a nonsignificant location effect (P value = 0.834), and highly significant cultivar and treatment effects (P values < 0.001). The majority of the cultivar differences observed were due to BD, as only two KO plants showed flower abscission. Since KO showed little flower abscission (Fig. 4), BD and KO were analyzed separately.

The nominal logistic model for KO was not significant (P value = 0.518). The nominal logistic model for BD was significant (P value = 0.022), with no evidence for lack of fit (P value = 0.680). The treatment effect was significant (P = 0.008) whereas the location and the location x treatment interaction effects were not. Four out of fifteen BD control plants did not flower within 70 days and were classified as having abscised flowers. Treatments wk 5-6, and wk 7-8 had the highest rate of flower abscission, (Fig. 4 and Table 9). The odds of BD plants from treatment wk 5-6 having more than two shoots with abscised flowers was greater than those of plants under continuous control conditions, wk 1-2, and continuous stress treated plants. The odds of a plant from treatment wk 7-8 having more than two shoots with abscised flowers were significantly greater than wk 1-2 and the continuous stress treatments (Fig. 4 and Table 9).

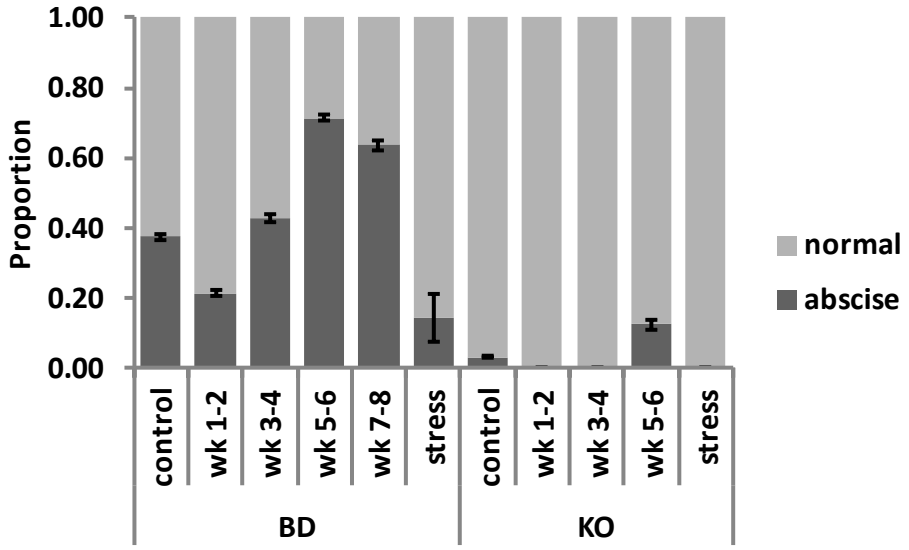

Fig. 4. Proportion of plants with more than two shoots abscising flowers over treatment for 'Belinda's Dream (BD) and 'RADrazz' (KO). "abscise" indicates having more than two shoots per plant with abscised flowers.

- Error bars represent one standard error of the probability.

Table 9. Significance of odds ratios for plants of 'Belinda's Dream' (BD) having more than two shoots with abscised flowers across all treatment combinations.

	Control	Week 1-2	Week 3-4	Week 5-6	Week 7-8	Stress
Control	-	NS[z]	NS	+	NS	NS
Week 1-2		-	NS	**	*	NS
Week 3-4			-	NS	NS	+
Week 5-6				-	NS	**
Week 7-8					-	**
Stress						-

[z], NS, +, *, **: Nonsignificant or significant at P value ≤ 0.1, 0.05, or 0.01 respectively.

Interestingly, the odds of a continuously stressed plant having two or more abscised flowers was significantly lower than the odds of plants from treatments wk 3-4, wk 5-6, and wk 7-8. BD flowers were more susceptible to flower abscission from a high temperature shock after reaching the visible bud stage (Fig. 4).

3.4.3 Time to flower

Based on Levene's test for equal variance, there was evidence for unequal variance in the days to flower between the two locations (P value = 0.023). College Station had greater variance in the number of days to flower. Days to flower had a standard deviation of 6.9 in College Station and 4.9 in Overton. Data was combined across locations regardless of unequal variance. For the same reasons as above, the two cultivars were analyzed separately. Only the main effects of location and treatment were significant for BD, and all effects were significant for KO (Table 7).

Plants from BD flowered five days earlier in Overton than in College Station. Plants of both BD and KO grown under continuous stress temperatures flowered in the least amount of days (Fig. 5). BD plants from treatment wk 7-8 took the longest to flower, whereas plants from treatment wk 5-6 flowered in the same amount of time as plants subjected to continuous stress (Fig. 5A). KO plants from treatment wk 1-2 took the longest to flower but not longer than control treated plants. Excluding continuous stress treated plants, KO plants from treatments wk 3-4 and wk 5-6 flowered in the least amount of days. Wk 5-6 plants were not different from the control (Fig. 5B).

The significant location x treatment interaction in KO explained nearly 10 % of the variation whereas the location and treatment effects explained 40 and 43 % of the

variation respectively. Apart from the control and wk 3-4 treatment respectively, flowering 7.3 and 3.1 days earlier in Overton, KO showed no difference in the number of days to flower between locations for the remaining treatments. KO control treated plants in College Station all flowered between 39 and 44 days, whereas in Overton the plants flowered between 29 and 37 days. The trend was similar for treatment wk 3-4 where plants in College Station flowered between 32 and 43 days and plants from Overton flowered between 27 and 36 days.

Since there was no evidence for unequal variance for thermal units accumulated up to flowering (TU1 and TU2), the data was combined over the two locations. For BD, the main effects of location and treatment but not the interaction was significant based on both equations (Table 7). All model effects were found to be significant for TUs accumulated for KO based on both equations (Table 7). The significant location x treatment interaction found for KO resulted from control and wk 3-4 treated plants in College Station accumulating more TUs than the same treatment in Overton. The interaction term accounted for 6 % with TU1 as response variable and 14 % with TU2 as response variable. It has to be taken into account that the model for TU2 had a lower coefficient of determination (adjusted R^2 0.80 vs. 0.59) than the model for TU1.

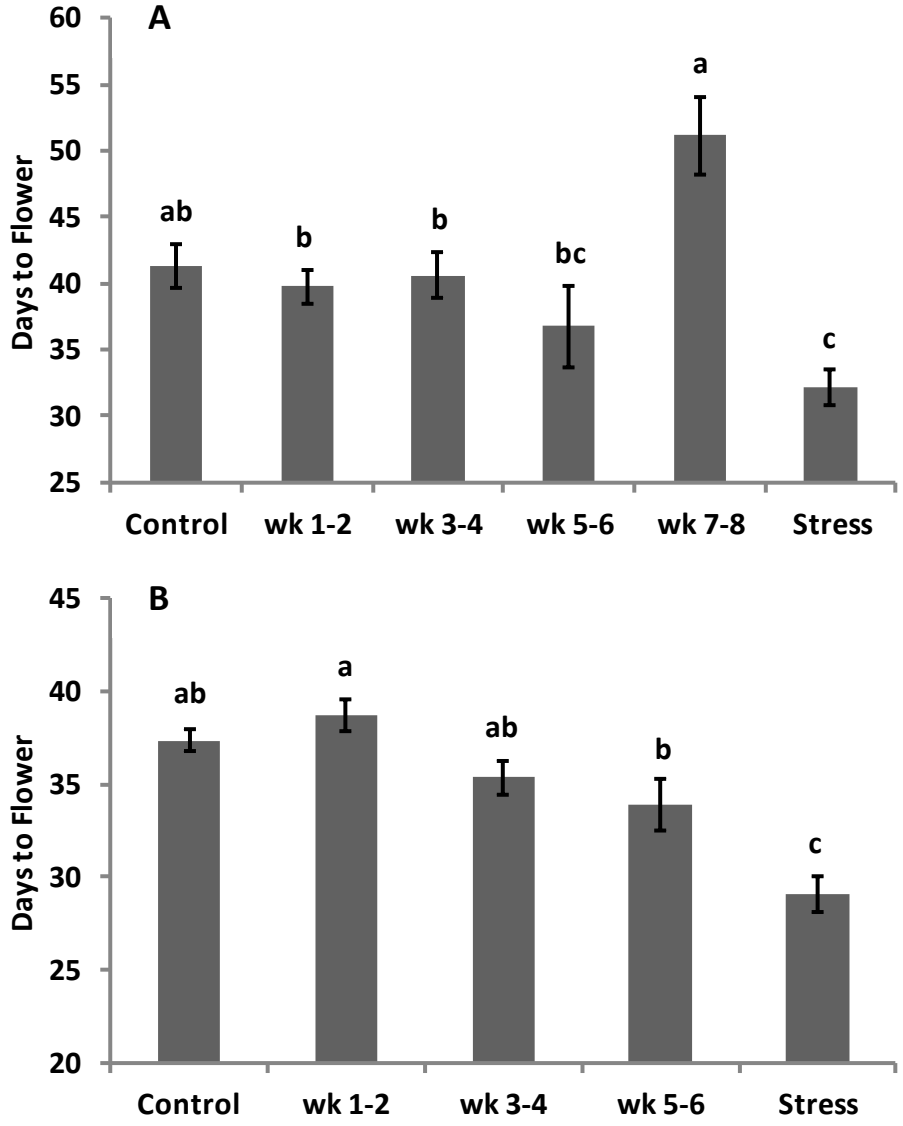

Fig. 5. Number of days to flower for 'Belinda's Dream' (BD) (A), and 'RADrazz' (KO) (B) at each treatment combined over both locations.

- Error bars represent one standard error of the mean.
- Treatments not connected by the same letter are significantly different at, $\alpha = 0.05$, with Tukey's adjustment.

Based on TUs accumulated from Eq. [1], plants subjected to continuous stress accumulated more hours than plants under control conditions for both cultivars. For both cultivars, TU1 under stress conditions grouped in the group accumulating the most TUs (Table 10). No differences in TU1 were seen among two week treatments for BD. Two week treatments of KO plants had wk 1-2 accumulate the greatest TUs followed by wk 3-4 and then wk 5-6.

The nonlinear TU accumulation based on Eq. [2] resulted in no difference in TU2 between control and continuous stress treated plants for both BD and KO (Table 10). BD plants from treatment wk 7-8 had the greatest TU2, whereas none of the other treatments were significantly different from each other. KO plants from treatment wk 1-2 had the greatest TU2, whereas none of the remaining treatments were significantly different from each other.

3.4.4 Number of nodes to flower

Data from both locations were combined as the variances were similar. A combined analysis revealed only the cultivar effect to be significant with KO (9.1 nodes) having fewer vegetative nodes to flower than did BD (10.7 nodes). The heat stress treatments did not affect the number of vegetative nodes produced to the flower.

Table 10. Thermal units accumulated (± 1 standard error) for 'Belinda's Dream' (BD) and 'RADrazz' (KO) across both locations for each treatment.

	BD		KO	
Treatment	[z]TU1	TU2	TU1	TU2
Control	649 ± 29 c[y]	649 ± 27 b	590 ± 10 c	587 ± 9 b
Week 1-2	789 ± 24 ab	681 ± 22 ab	773 ± 15 a	664 ± 13 a
Week 3-4	802 ± 31 ab	694 ± 29 ab	702 ± 15 b	595 ± 13 b
Week 5-6	674 ± 55 bc	610 ± 51 b	602 ± 22 c	555 ± 20 b
Week 7-8	899 ± 52 ab	836 ± 47 a	-	-
Stress	883 ± 24 a	643 ± 22 b	799 ± 15 a	573 ± 14 b

- [z], TU1 and TU2 refers to thermal unit accumulated in hours calculated based on following equations: $TU1 = \sum_{j=1}^{r} max\left[\left(T_j - T_{b1}\right), 0\right]\Delta t_j$ and $TU2 = \sum_{j=1}^{r} max\left[T_i - T_{b2} + k\left(T_j - T_i\right), 0\right]\Delta t_j$. Where r is the number of days to flower, T_j is the average air temperature (ºC) over period j, Δt_j is the length of time period j (24 h), T_{b1} and T_{b2} is the base temperature (5.2 and 9.5 ºC), T_i is the temperature where thermal units are not linearly accumulated (25.6 ºC), and k is the ratio of the slope for the regression line at $T_j > T_i$ and $T_j < T_i$ (0.47) (Pasian and Lieth, 1994; Steininger et al., 2002).
- [y], Levels not connected by the same letters are significantly different at $\alpha \leq 0.05$, with Tukey's adjustment. Comparisons are made among treatments within cultivar.

3.5 Discussion

Flower dry weight and flower abscission was most affected at the visible bud stage which corresponds to stress treatments during weeks 5-6 and 7-8 for BD and weeks 3-4 and 5-6 for KO. The proportionate reduction in flower size between BD and KO was the same when plants were subjected to continuous stress. Fading of flower color was observed but not recorded when plants were stressed at the visible bud stage for both cultivars.

'Madelon' roses subjected to drought stress was most affected at the stamen formation prior to carpel formation stage of development (Chimonidou-Pavlidou, 2004), thus slightly earlier than the visible bud stage. 'Jaguar' rose seedlings were most susceptible to reduction in anthocyanin preceding pigmentation of flower buds (Dela et al., 2003), thus later than the visible bud stage. Rose flowers were more sensitive to high growing temperatures after the visible bud stage and flower size was reduced in a quadratic fashion with increasing growing temperatures (Shin et al., 2001). Literature suggests that flower size and quality are most affected by abiotic stress factors after the visible bud stage of development, and the results of flower size and abscission presented is in agreement with current literature. Different from Shin et al. (2001) who subjected plants to different constant growing temperature after specific developmental stages have been reached, predetermined time periods for treatments were used. Dela et al. (2003) subjected plants to short 1 - 3 day heat shock treatments. Close tracking of flower developmental stages and short heat shock treatments would provide a good comparison

of short heat shock treatments at sensitive stages of development on flower size and abscission.

Both BD and KO flower sizes were reduced by 70 % when grown under continuous high temperature stress. The low amount of flower abscission for BD plants grown under continuous stress is evidence of BD's ability to adapt to growing under high temperatures. The flower abscission response when stressed at the visible bud stage was probably due to the shock of being moved from the control environment into the stress environment at that critical time. Plants adapt to their environment, *Nerium oleander* plants grown at 45/32 ºC (day/night) had a different membrane lipid composition than those grown at 20/15 ºC (Raison et al., 1982). The membranes of plants grown under high temperature were less fluid providing integrity under elevated temperatures. Thus even though the flowers of continuously grown BD plants were subjected to the high temperatures during the visible bud stage the plants had time to respond to growing under elevated temperatures.

The 8 % difference in flower size reduction between BD and KO when stressed at the critical time was not different from each other. A larger sample size would be required to investigate whether flower size reduction when stressed at the visible bud stage is different between BD and KO. Both BD and KO are well adapted and carry the Earth-Kind™ designation, which means that they perform well in the landscape under a wide range of conditions (Texas a&M University System., 2013). Differences in flower size reduction between well adapted and not so well adapted cultivars might prove to be greater than we observed between these two adapted cultivars.

Larger flowers are associated with cooler growing conditions. Shin et al. (2001) reports that flowers of 'Kardinal' produced at 15 ºC was 3 g compared to less than 2 g for temperatures above 24 ºC. The increase in flower size between the control and continuous stress plants in our study could in part be attributed to the increased number of days to flower for control plants in combination with reduced photosynthesis at the higher temperature. Jiao and Grodzinski (1998) reported a significant reduction in photosynthesis and carbohydrate export at 40 ºC compared to 15 ºC in 'Samantha' roses.

Both cultivars flowered in the least amount of time under continuous stress conditions. Developmental rates of rose shoots are heavily influenced by air temperature. Pasian and Lieth (1994) report a positive linear relationship between the rate of development and air temperature for all stages of shoot development, with the exception of bud break after pruning. A reduction in the number of days to flower compared to the control treatment would be expected for both cultivars.

It is expected that the number of TUs accumulated to reach a certain stage of development remain fairly constant within a cultivar. The differences in TU1 between control and continuous stressed plants for both BD and KO indicated that a linear accumulation of TUs for the temperatures used was not appropriate. No differences in TU2 were seen between control and continuous stressed plants for either cultivar suggesting that a nonlinear accumulation of TUs occur for both BD and KO. Pasian and Lieth (1994) reported that TU accumulation could not reliably predict the period from pruning until bud break, not taking the time to bud break into account in this experiment likely added to the variation. The linear model for TU accumulation used by (Pasian and

Lieth, 1994) remains valid within the temperatures experienced during their experiment where the maximum temperature was 29.8 ºC. Steininger et al. (2002) reports that TU accumulation is cultivar dependent as they found no nonlinearity for miniature rose 'Red Sunblaze' even though it was grown at 35 ºC.

An increase in the days to flower and TUs accumulated for BD plants from treatment wk 7-8 is contradictory to what was expected. It is possible that early flower bud abscission was not detected and plants continued to grow vegetatively for a longer period of time until they reached flowering. This is unlikely to have been the cause for the delayed flowering as an increase in the number of vegetative nodes would be expected under such conditions. None of the treatments had an effect on the number of vegetative nodes. An increase in the time to flower for plants of chrysanthemum 'Bright Golden Age' (*Dendranthema grandiflora*) was reported by Karlsson et al. (1989) when plants at the visible bud stage of development were moved from cooler growing conditions to 30 ºC conditions.

The only difference in the number of nodes to flower was the cultivar difference. KO flowered in less time and produced less vegetative nodes to flower than did BD (9.1 vs. 10.3). Marcelis-Van Acker (1995) concludes that the number of vegetative nodes on rose shoots are determined during the formation of the axillary buds, thus the temperature regime the parent shoots are exposed to could affect the number of vegetative nodes. All the plants from our experiment were grown under the same conditions prior to the onset of the experiment and the axillary buds were presumably

formed under the same conditions. The treatments not having an effect on the number of vegetative nodes was as expected.

3.6 Conclusions

Flower quality and abscission are most sensitive to high temperature stress after flowering shoots have reached the visible bud stage. Lower flower abscission rates for BD under continuous stress conditions provided evidence of BD's ability to acclimate to high temperature conditions, and that the increased rates in flower abscission were probably due to a high temperature shock. Based on near zero flower abscission, KO may be ranked as more tolerant to a high temperature shock. Evaluating garden rose flowering response after a high temperature shock could be used in screening roses for high temperature tolerance.

Plants flowered faster under continuous stress conditions compared to the control conditions. No differences in TU2 accumulated between control and continuous stressed plants of either cultivar suggest that there is a nonlinear accumulation of TUs and that the stress treatment was severe enough to exceed optimum temperatures of development for both cultivars. Results presented also suggest that a nonlinear TU accumulation model is more appropriate than a linear TU accumulation model. None of the treatments had any effect on the number of vegetative nodes to flowering for either BD or KO. Selecting rose seedlings with a high minimum T_i could prove useful in breeding towards roses with better performance under high temperature conditions.

Although the differences in flower size reduction between BD and KO shoots subjected to a high temperature shock at the critical stage were not significant,

differences in flower size reduction between cultivars could be a useful indicator of a cultivar's ability to maintain flower quality during periods of high temperature stress. Plants showing major reductions in flower size would be likely more heat susceptible.

Evaluating rose plants in the field is costly and ties up land and resources for several years as it takes a number of years to properly evaluate a new seedling. Subjecting plants to a high temperature shock with flower buds at the visible bud stage of development could be the first step in selecting against the most high temperature susceptible roses. Propensity towards flower abscission and reduction in flower size in combination with measurements evaluating the maximum temperature where plant development starts to decline could be used to quantify garden rose high temperature susceptibility and hopefully add to breeding progress for better adapted garden roses.

CHAPTER IV

DEVELOPMENT OF TWO RAPID SCREENING METHODS FOR SELECTION AGAINSTHIGH TEMPERATURE SUSCEPTIBLITY IN GARDEN ROSES

4.1 Synopsis

The decline of garden rose sales over the past 20 years can, in part, be attributed to the lack of material adapted to a wide range of landscapes, which includes adaptation to high temperature stress. Current methods for evaluating high temperature susceptibility in garden roses are based on field observations which are time consuming and subjected to ever changing environmental conditions. A series of experiments was conducted to optimize protocols and compare the use of chlorophyll fluorescence (CFL) and cell membrane thermostability by way of electrolyte leakage (MTS) as methods to screen for high temperature susceptibility. Immature leaves proved better than mature leaves for both CFL and MTS measurements, using either detached leaf or whole plant stress assays. MTS measurements on immature leaves stressed in a water bath at 50 ºC for 45 min proved most consistent in separating rose clones based on high temperature susceptibility. Stressing actively growing plants with flower buds of 2 mm in diameter in a heat chamber at 44 ºC for three hours resulted in increased flower abscission and leaf necrotic lesions on a more susceptible clone when compared to those that were heat tolerant. Combining MTS measurements from immature leaves stressed in a water bath with the flower abscission and leaf necrosis responses ten days after stress in a heat

chamber could be the first step to screen and select against the more susceptible clones in a garden rose breeding program. Power analysis suggests that the proposed MTS protocol would be efficient in detecting differences between clones when the difference in electrolyte leakage is greater than 10 percent.

4.2 Introduction

Overall sales of garden roses have been declining over the past 20 years in the U.S. due in part to the lack of widely adapted cultivars to heat, drought and salt stress in landscape environments (Byrne et al., 2010). Conversely, an increase in sales of shrub-type rose cultivars which are widely adapted to stress conditions such as cold, high temperature and drought, e.g. Knock Out®, has occurred. (Hutton, 2012).

High temperature stress is a major limiting factor to growing agronomic and horticultural crops worldwide (Wahid et al., 2007). High temperature stress, the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid et al., 2007), is both a factor of intensity and duration of elevated temperature. High temperature tolerance is a plant's ability to grow and produce an economically viable yield under such conditions (Wahid et al., 2007).

Elevated production temperatures resulted in decreased flower quality of greenhouse grown cut rose flowers by reducing size (Shin et al., 2001), color (Dela et al., 2003), and post harvest life (Marissen, 2001; Moe, 1975) . To our knowledge, little is known about the effect of high temperature stress or how to efficiently evaluate high temperature susceptibility on garden roses. Currently, no method, apart from field

observations, has been developed for phenotyping high temperature susceptibility in garden roses. Yield of garden roses was quantified at the Texas A&M Rose Breeding Program as landscape performance, and was rated on a 1 - 5 scale. Landscape performance was influenced by the ability of the rose to maintain healthy foliage and a high percentage of the plant perpetually covered in blooms.

The earliest work on high temperature susceptibility of plants was performed by Sachs (1864) involving stressing leaves in a water bath followed by scoring the extent of necrotic lesions in the days to follow. The photosynthetic apparatus in plants is sensitive to temperature (Berry and Björkman, 1980). High temperature stress in plants is associated with reductions in photosynthetic activities and has been verified for members of the Rosaceae, including red raspberry (*Rubus idaeus* L.), by recording the net photosynthetic rate of leaves at different temperatures (Fernandez and Pritts, 1994). Chlorophyll fluorescence (CFL) is a nonintrusive measurement (Krause and Weis, 1991) used as a physiological parameter which correlates with thermo tolerance to both high (Camejo et al., 2005; Weng and Lai, 2005; Yamada et al., 1996) and low temperatures (Stoddard et al., 2006).

When light energy enters the cell, and drives photochemistry, it is dissipated as heat, or is reemitted as fluorescence. Chlorophyll fluorescence is usually measured as the ratio of variable fluorescence (F_v) to maximum fluorescence (F_m) (Krause and Weis, 1991). F_0 is the fluorescence emitted when all the reaction centers in photosystem II are open. F_m is the fluorescence emitted when all the reaction centers in photosystem II are closed. F_v is the maximum variable fluorescence ($F_v = F_m - F_0$) (Krause and Weis,

1991). A reduction of the F_v/F_m is expected under high temperature stress conditions. The nonintrusive nature of taking chlorophyll fluorescence measurements makes CFL a desirable approach to screen large numbers of individuals within breeding populations (Srinivasan et al., 1996).

Bilger et al. (1984) reported a significant correlation (r = 0.87) between the temperature resulting in 50 % necrosis and the temperature at which dark fluorescence (F_0) starts to increase. While CFL has not been investigated to our knowledge for high temperature tolerance in roses, CFL has been successfully used as an indicator of low temperature tolerance among thirteen rose genotypes with varying degrees of tolerance. Hakam et al. (2000) found that the slope of F_v reduction among different genotypes accurately grouped genotypes as very resistant, resistant, or sensitive. These F_v groupings coincided with visual scores of necrosis from chilling injury.

High temperature injury can result in heat-induced loss of the semi-permeability of the plasma-membrane, the tonoplast, or other membranes within the cell (Berry and Björkman, 1980). Cell membrane thermostability (MTS), which makes use of a conductivity test to measure the amount of electrolyte leakage from leaf disks, has been used as an indicator of high temperature tolerance in various crops. MTS has been successfully used as an indicator of high temperature tolerance on field crops such as wheat (*Triticum aestivum* L.) (Ibrahim and Quick, 2001a), twenty different species of vegetables (Kuo et al., 1993), tomato (*Solanum lycopersicum* L.) (Camejo et al., 2005), food legumes (Srinivasan et al., 1996) including cow-peas (*Vigna unguiculata* L.)

(Thiaw and Hall, 2004), and ornamental plants such as chrysanthemum (*Dendranthema* x *grandiflora*) (Wang et al., 2008; Yeh and Lin, 2003).

Srinivasan et al. (1996) compared CFL and MTS as methods for phenotyping high temperature tolerance on four different food legumes and found both methods to be successful, with the correlation between CFL and MTS ranging between 0.57 and 0.87. Camejo et al. (2005) reported both CFL and MTS as successful in distinguishing between a high temperature tolerant and susceptible tomato lines. The differences in protocols using either CFL or MTS as indicators of stress tolerance for various crops are usually based on stress temperature, duration, and the age of plant tissue. These variables, in combination with sound experimental design, have to be optimized for each crop and technique before they can be applied effectively.

Currently, no rapid laboratory screening method has been developed for phenotyping high temperature susceptibility in garden roses. The objective was to develop a rapid screening technique for phenotyping garden roses, specifically cultivars recommended for Texas landscape use, for high temperature susceptibility and to compare the efficacy of CFL and MTS as indicators of high temperature tolerance. Guidelines on the power of experiments applying the proposed protocol are presented.

4.3 Materials and methods

4.3.1 Plant material

A series of experiments was conducted over a three year period beginning April, 2010 and ending December, 2012. Both commercial cultivars and breeding lines

(referred to as clones from here forward) of garden roses and both field and greenhouse grown plants were used. Field grown plants were well established and maintained under ambient conditions on the Texas A&M University Horticulture Research Farm or in raised beds in a trial rose garden on campus both in College Station, TX.

Greenhouse grown plants were kept at temperature set points 25/20 ºC day/night, however, temperatures increased to as high as 34 ºC during summer days. Temperature was controlled by a pad and fan cooling system in the summer months and by natural gas heaters in the winter months. All greenhouse plants were grown in a bark-based, soilless media (Fafard 52 Mix, Conard Fafard Inc., Agawam, MA)

Greenhouse plants were either obtained from The Antique Rose Emporium, Inc., Brenham, TX, as well-established plants in 7.8 L plastic pots or propagated from stem cuttings. Stem cuttings were two node vegetative cuttings from recently flowered shoots. The basal end of each cutting was dipped in Rootech Cloning Gel™ (Technaflora Plant Products Ltd., Mission, British Colombia, Can.), and rooted under mist using 1 perlite : 1 Fafard 52 potting mix for support. Frequency and duration of misting was adjusted depending on ambient conditions. Once rooted, cuttings were potted into 1.8 L pots and later transplanted into 7.8 L plastic pots. Pests were controlled as necessary. The plants were irrigated and fertilized with each irrigation as required with a 200 $mg \cdot L^{-1}$ 20N – 3.4P – 16.6 K liquid fertilizer except during the months of Dec. to Feb. when 200 $mg \cdot L^{-1}$ 15N – 5.4P – 14.1 K was used. Periodically and especially in warmer months, 5.15 $g \cdot L^{-1}$ Sequestrene 138 (6% iron chelate) (Becker Underwood, Inc., Ames, IA) was applied as a drench to prevent iron deficiency.

4.3.2 Detached leaf stress conditions

When detached leaves were used for CFL and MTS experiments, they were either the most recently fully expanded five-leaflet leaves (mature) or unfolded leaves (immature) directly below a folded leaf on actively growing shoots. To avoid diurnal effect, all leaves were sampled in the mornings before 1000 $_{HR}$ and placed in small, sealable clear plastic bags with a moist paper towel (to maintain high humidity), with the majority of the air vacuumed out.

For CFL measurements the leaves were subjected to high temperature stress while remaining in the plastic bags. CFL measurements were recorded with a HANDY-PEA, chlorophyll fluorescence system (Hansatech Instruments Ltd., Norfolk, U.K.). Prior experimentation verified a 30 min dark adaptation period, and a 1 s saturation pulse with light intensity 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ optimal for recording CFL on garden roses. Fluorescence measurements recorded included F_0 and F_m and F_v/F_m was computed. CFL was recorded 30 min post stress treatments.

The MTS protocol was performed in 25 mm x 95 mm flat bottom glass culture tubes. Preliminary experimentation verified that using ten to fifteen 9.5 mm diameter leaf disks in 10 mL of distilled water was optimum for MTS measurements of roses. Some of the clones had small leaves, and in special cases, when not enough leaf tissue existed to harvest 15 disks, the leaflets were cut into 5.0 mm strips. Leaflets wider than 15 mm were cut along the midrib and subsequently cut into 5 mm strips. After collection, leaf disks were washed and rinsed three times with distilled water and subjected to high temperature stress treatments. Following treatment, culture tubes

containing the leaf disks were placed in a water bath set at 25 ºC for 5 min and then moved to a rotary shaker (60 rpm) at room temperature for 24 h, after which the first of two conductivity readings (EC1) was recorded. The tubes were then autoclaved for 20 min (121 ºC, 1.2 $kg \cdot cm^{-2}$). When cooled to 25 ºC, the second conductivity reading (EC2) was taken. MTS was expressed as the reciprocal of relative leakage: MTS = (1 - EC1/EC2) (Ibrahim and Quick, 2001b). Electric conductivity was measured by vortexing the test tubes for 1 s and pipetting 60 µL onto a Horiba B-173 Compact Conductivity Meter, (Horiba Ltd., Edison, NJ). The average of three measurements was recorded per sample. Unless otherwise stated, experiments were conducted using a randomized complete block design.

4.3.3 Whole plant stress conditions

High temperature stress treatments were conducted on whole plants in a greenhouse during the summer of 2011. Stress treatments consisted of shutting off the pad and fan cooling system and opening the shade in the greenhouses until temperatures reached 45 ºC then placing plants in the greenhouse for various durations. Bench temperatures in the greenhouse where stress conditions were imposed reached as high as 53 ºC and had a mean of 51 ºC during stress conditions. The maximum temperature recorded in the control greenhouse was 36 ºC and the mean temperature during the course of the experiment was 33 ºC. The light intensity in the stress greenhouse was nearly three times that of the light intensity in the control greenhouse. The mean photosynthetic active radiation (PAR) in the stress greenhouse was 890 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to 301 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the control greenhouse. The maximum PAR recorded

during the stress period was 1531 μmol·m^{-2}·s^{-1} compared to the maximum PAR for control conditions of 424 μmol·m^{-2}·s^{-1}.

Subsequently, to enable year-round whole plant stress treatments, a high temperature stress chamber was constructed by converting a 2.13 m x 2.74 m x 2.00 m (L x W x H) walk-in refrigerator with a time controller for lights, an environmental controller, heating element, and humidifier. Fans were installed for air circulation. The heat chamber construction allowed for the control of temperature within in the range 20 – 50 ºC ± 1 ºC, and relative humidity (RH) within the range of 10 - 60 % ± 3 %. Both fluorescent and incandescent bulbs were used as light sources and resulted in PAR of 25 μmol·m^{-2}·s^{-1} at plant canopy level, measured with a ceptometer (Accupar LP-80, Decagon Devices, Inc., Pullman, WA).

4.3.4 Experiment 1: Preliminary investigation of detecting differences between clones

Five clones observed to have varying landscape ratings (unpublished data) under the hot and humid field conditions in College Station, TX were used including 'Belinda's Dream' (BD), 'Basye's Blueberry' (BB), 'Caldwell Pink' (CP), 'Marie Pavié' (MP), and 'Old Blush' (OB). From four plants (replicates) of each clone, two mature leaves (subsamples) were harvested per replicate. The four replicates were grown in separate beds in the trial garden. CFL was recorded prior to stress treatment then leaves were stressed in a water bath at 50 ºC ± 1 ºC for 60 min (Kuo et al., 1993), followed by CFL measurement 30 min post stress.

Next, to optimize duration of water bath high temperature stress (50 ºC ± 1 ºC), six mature leaves (subsamples) from three plants (replicates) of field grown BB and BD

were harvested. The three stress duration treatments (15, 30, or 60 min) were followed by CFL measurements.

Finally, to compare efficacy of CFL vs. MTS, eight mature leaves (subsamples) from two plants (replicates) from field grown plants of J06A (high temperature tolerant breeding line from the TAMU Rose Breeding Program) and 'Vineyard Song' (VS) (high temperature susceptible) were subjected to 45 min stress in a water bath set at 50 ºC. CFL was recorded on each leaf pre-and post stress treatment then leaves were prepared for MTS measurements.

4.3.5 Experiment 2: Improving resolution with clone selection, heat source, detached vs. whole plant, and leaf developmental stage

It was concluded from the first experiment that lack of differences among clones may have been due to the fact they were all of similar heat tolerance in landscape trials and that more diverse cultivars should be used. Two mature leaves (subsamples) from five plants (replicates) grown in 7.8 L pots of three clones: BD, Knock Out® rose 'RADrazz' (KO), and 'Sir Thomas Lipton' (SL), were stressed in a water bath set at 50 ºC for 45 min and CFL measurements were recorded. KO has superior landscape performance in North-central Texas over BD and SL (Mackay et al., 2008)

Next, four plants (replicates) of the same three clones (BD, KO, and SL) grown in 7.8 L pots were subjected to control or high temperature stressed conditions in the greenhouse. Control plants were kept under ambient conditions and stressed plants were moved to the adjacent greenhouse where stress conditions were imposed. CFL measurements were recorded prior to stress conditions and then hourly for three hours on

two mature leaves (subsamples) per plant. Plants were allowed to recover overnight and CFL was recorded on the same leaves again at 0920 $_{HR}$. CFL measurements from different subsamples were averaged over individual plants.

Finally, immature leaves of eight plants (replicates) of BD, KO, and SL grown in 7.8 L pots were subjected to stress conditions in the greenhouse and CFL was recorded on one immature leaf per plant at five times including: (1) prior to the onset of stress; (2) 40 min into stress conditions; (3) 110 min into stress conditions; (4) 60 min after 110 min of stress; and, (5) in the morning at 0920 $_{HR}$ after plants had time to recover from treatment overnight.

4.3.6 Experiment 3: Optimization of duration of heat chamber treatment

Because the greenhouse was not deemed a year-round way to impose heat treatments, the heat chamber was tested in this experiment. The objective was to determine whether a three or five hours of high temperature stress in a heat chamber was sufficient for detecting differences between clones with known differences in landscape performance. Four plants (replicates) in 1.8 L containers from clones J06A, VS, and SL were stressed in the heat chamber set at 42 ºC and 35 % RH. Two immature leaves (subsamples) on each plant were labeled prior to the experiment and CFL was measured on each leaf before the plants were subjected to high temperature stress conditions. Subsequently, a separate set of plants were subjected to a five hour heat chamber treatment. The plants were moved from the greenhouse at 0800 $_{HR}$ and moved to the heat chamber. All labeled leaves were detached immediately after the stress treatment and processed for CFL measurements followed by MTS measurements on the same leaves.

4.3.7 Experiment 4: Comparing efficacy between CFL and MTS, and differences on whole plant level after high temperature treatment

This experiment consisted of two phases, with the first directly comparing CFL and MTS, and the second evaluating differences in response to high temperature shock on a whole plant level between clones with observed differences in the field. Ten plants of J06A, J06B, 'Sweet Chariot' (SC), and VS were grown under greenhouse conditions in 1.8 L pots. J06B is a tolerant breeding line similar to J06A, whereas SC is similar in susceptibility to VS. When established, plants were pruned back to stimulate and synchronize re-growth. Due to growth rates of the clones and available space in the heat chamber, the experiment was conducted in two groups: J06B with SC first and then J06A with VS. The first group was pruned ten days prior to the second group.

When at least five immature leaves on seven plants (replicates) of J06B and SC were present, two immature leaves per plant (subsamples) were harvested and used in a detached leaf assay. High temperature stress was administered in a water bath at 50 ºC for 45 min. Post stress CFL was recorded followed by MTS on the same leaves. The following day two immature leaves (subsamples) on the same plants were labeled and the plants were subjected to high temperature stress in the heat chamber set at 44 ºC and 50 % RH for 3 hours. After the stress treatment the plants were removed from the heat chamber and the labeled leaves were detached immediately and processed for measurements. CFL was recorded on each leaf followed by MTS on the same leaves. When seven plants from J06A and VS reached the correct stage of growth, they were subjected to the same experimental procedure as the first group.

Following the heat chamber treatment all J06B and SC plant were returned to the greenhouse. Three shoots with a terminal flower bud approximately 1 - 2 mm in diameter were labeled for visual evaluation. Three shoots on three plants of J06B and SC that were not subjected to the heat chamber treatment were also labeled for comparison.

All plants were evaluated ten days post heat chamber treatment. The total number of shoots with at least one aborted flower and the number of shoots with no visible sign of flower abscission was recorded on each plant (Fig. 6A). The presence or absence of leaf necrosis was scored on the tenth day for each of the labeled shoots (Fig. 6C).

4.3.8 Experiment 5: Optimized rapid screening protocol

The objective of this experiment was to use the optimized conditions from the previous experiments to evaluate differences in high temperature tolerance between J06B and VS. Ten well established plants (replicates) in 1.8 L pots were used and plants were pruned to stimulate and synchronize re-growth. Two immature leaves (subsamples) from each plant were harvested and prepared for MTS measurements. The tubes containing the leaf disks were subjected to stress in a water bath set at 50 ºC for 45 min.

4.3.9 Power and sample size

Power and sample size calculations were performed to aid in the development of future studies. Calculations were based on the smallest significant differences in MTS detected between clones when immature leaves were stressed in a water bath. The residual variance (σ^2 – statistical analysis below) of five detached leaf experiments using the suggested protocol (results of all experiments not presented) was averaged resulting

in σ of 0.0483 used as the residual standard deviation in power calculations. The block variance (σ^2_B) for the same five experiments was averaged resulting in σ_B of 0.0873 used in power calculations. Power calculations were performed for a randomized complete block design comparing 2, 4, 8, or 20 clones with the number of blocks ranging from 2 to 20. The significance level (α) was set at 0.05. Power analyses were performed to detect MTS differences of 0.05, 0.1, and 0.2 between clones. Power analyses were adjusted with Tukey's adjustment for multiple error testing. All power analyses were performed using Piface software (Lenth, 2006).

4.3.10 Statistical analysis

All statistical analyses were performed using JMP (version 9.0; SAS Institute Inc., Cary NC 1989 - 2010). Randomized complete block layouts with subsampling were analyzed by fitting a mixed least squares model. Experiments with repeated measurements on the same plants were analyzed by averaging over the subsamples and fitting an univariate repeated measures model. The unbalanced experiment was analyzed by fitting a mixed least squares model. The random effect of individual plants was included in the repeated measures and models where the design was unbalanced. Differences among fixed effects were investigated by Student's t-test pairwise comparisons and Tukey's honestly significant difference tests. Linear contrasts were performed to investigate significant two way interaction effects. The Pearson's correlation coefficient was calculated to evaluate the relationship between CFL and MTS where applicable. Nominal logistic regression was performed on binomial data and differences were evaluated based on odds ratios.

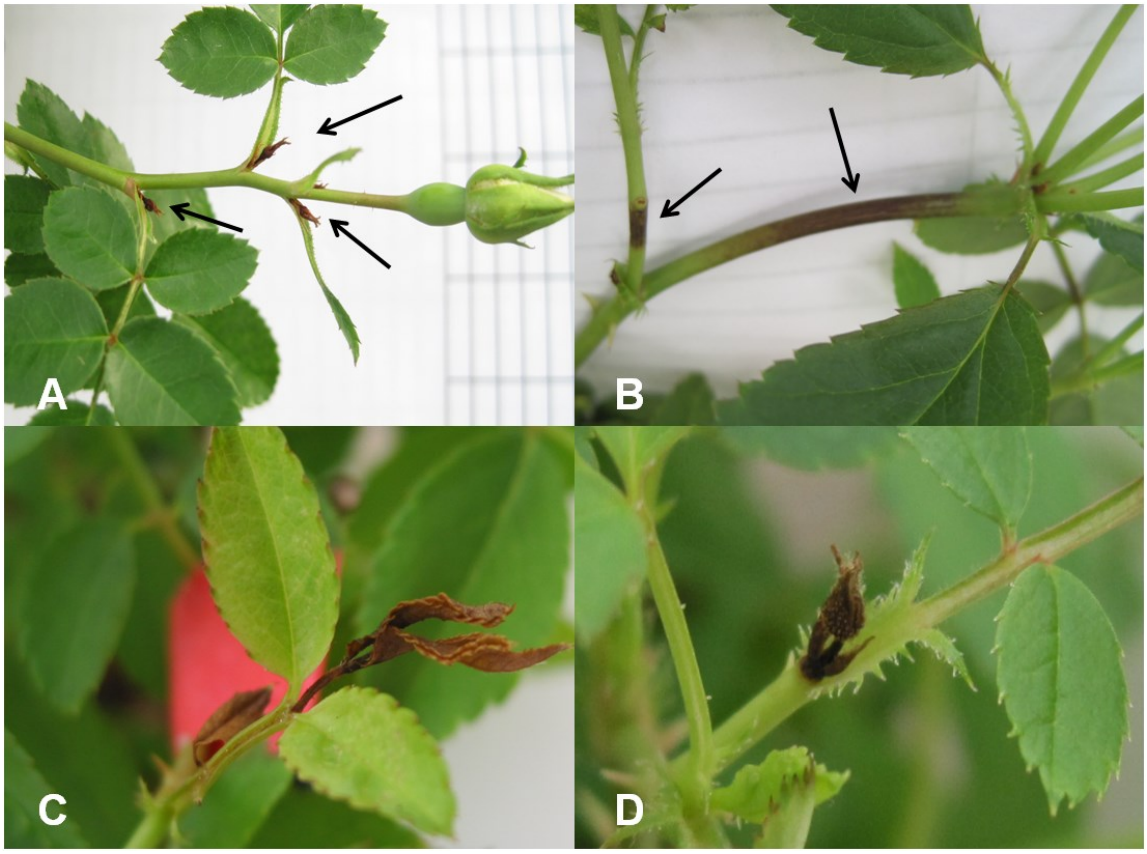

Fig. 6. Ten days post heat chamber treatment (44 ºC and 50 % RH), visual signs of high temperature stress was observed and recorded. Axial flower buds abscised (A), shoot burn showing clear signs of browning (B), leaf necrosis was mostly limited to immature leaves (C), and a number of terminal flowers abscised (D). Black arrows indicate: axial flower bud abscission (A), and browning of shoots (B).

4.4 Results

4.4.1 Experiment 1

No differences in CFL were detected among the five clones prior to the high temperature stress in the water bath, with the overall mean F_v/F_m for the five clones being 0.827. No significant differences in CFL post stress was detected among clones, with the overall mean F_v/F_m being 0.327. Although not statistically significant, the clones with the greatest difference in CFL between them were BD and BB (0.204).

When BD and BB were subjected to different stress durations, BD had a greater F_v/F_m at all three durations (Fig. 7). The greatest difference (0.315) between BB and BD was observed after 30 min of stress. It was concluded that a stress duration of at least 30 min and less than 60 min would be used for future experiments to achieve maximum separation between clones.

Subjecting mature leaves from J06A and VS to high temperature stress in a water bath revealed no differences between clones using CFL (Fig. 8A). MTS resulted in significant clone effect with VS having the greatest amount of electrolyte leakage (Fig. 8B). The MTS result was in accordance to landscape performance as VS appeared more susceptible than J06A to high temperatures in field evaluations in College Station.

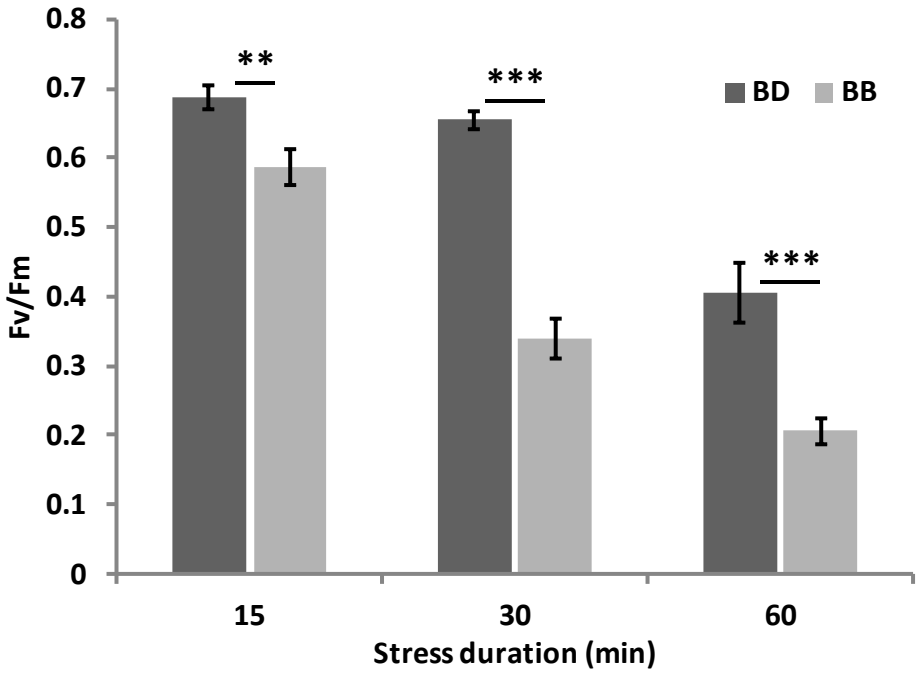

Fig. 7. Chlorophyll fluorescence measured as F_v/F_m on detached mature leaves of 'Basye's Blueberry' (BB) and 'Belinda's Dream' (BD) over three durations of high temperature stress in a water bath (50 ºC).

- Error bars represent one standard error of the mean.
- **, ***, Indicate significance of linear contrast between clones for each stress duration at $\alpha \leq 0.01$ and 0.001.

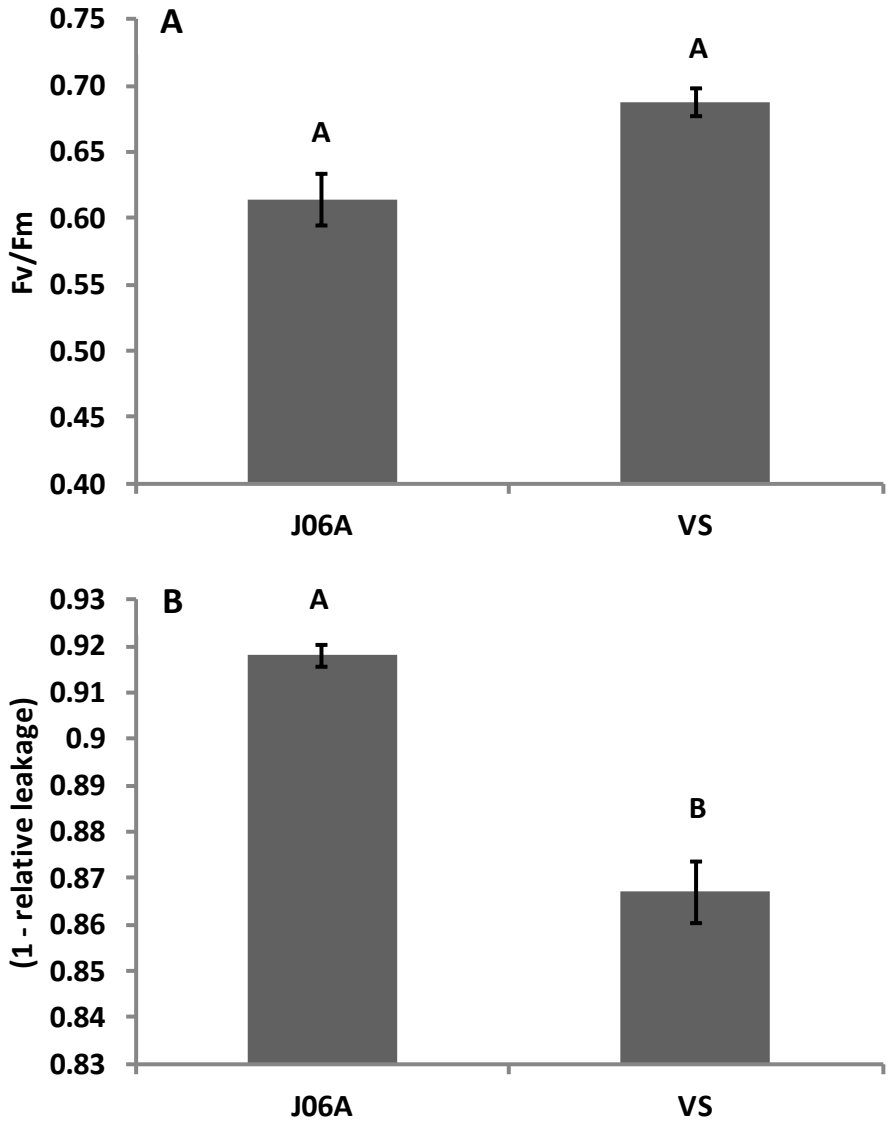

Fig. 8. Chlorophyll fluorescence measured as F_v/F_m (A) and cell membrane thermostability measured as 1 - relative electrolyte leakage (B) for garden rose clones 'Vineyard Song' (VS) and J06A using mature, detached leaves subjected to high temperature stress in a water bath (50 ºC for 45 min).

- Error bars represent one standard error of the mean.
- Clones not connected by the same letter are significantly different, $\alpha \leq 0.05$.

4.4.2 Experiment 2

Mature detached leaves of BD, KO, and SL were subjected to heat stress and no differences in CFL were detected among clones. The inability to detect differences based on detached leaf assays prompted whole plant stress assays.

The mature leaves of BD, KO, and SL subjected to high temperature stress under greenhouse conditions were monitored for CFL and showed significant time, treatment, and treatment x time effects (Table 11). No change in CFL over time occurred for plants in the control greenhouse (Fig. 9). Plants subjected to stress treatment showed a significant reduction in CFL and did not recover completely after an overnight period (Fig. 9). During this experiment it was observed that immature leaves on all three clones showed visible signs of stress such as wilting unlike the mature leaves that did not wilt.

No differences in CFL were detected on immature leaves of BD, KO, and SL prior to the onset of stress conditions in the greenhouse. CFL of immature leaves from plants subjected to stress under greenhouse conditions resulted in significant differences: clone, time, and clone x time effects (Table 11). After 110 min, all the immature leaves started showing signs of wilting making CFL measurements challenging. All clones showed a reduction in CFL during stress conditions and BD had the greatest reduction (Fig. 10). At no time was CFL different between KO and SL. The overnight recovery period allowed leaves of KO and SL to fully recover, whereas BD leaves did not show any recovery, pointing towards permanent injury of BD leaves (Fig. 10).

Table 11. F-ratio tests of fixed effects on chlorophyll fluorescence measured on mature and immature leaves of whole plants subjected to high temperature stress in the greenhouse.

Source of Variance	Mature leaves	Immature leaves
Clone	0.001^{NS}	16.371^{***}
Time	22.048^{***}	47.308^{***}
Clone x time	1.077^{NS}	11.839^{***}
Treatment	27.509^{***}	-
Clone x treatment	0.172^{NS}	-
Treatment x time	21.956^{***}	-

- ^{NS,} ***, Nonsignificant and significant at P value ≤ 0.001.
- Results were analyzed by fitting a univariate repeated measures model and included the individual plant as random effect.
- Clones included: 'Belinda's Dream', 'RADrazz', and 'Sir Thomas Lipton'.
- Measurements for mature leaves were taken over six time periods, and immature leaves were measured over five time periods.
- The treatment effect of mature leaves included high temperature stress greenhouse and control conditions greenhouse.

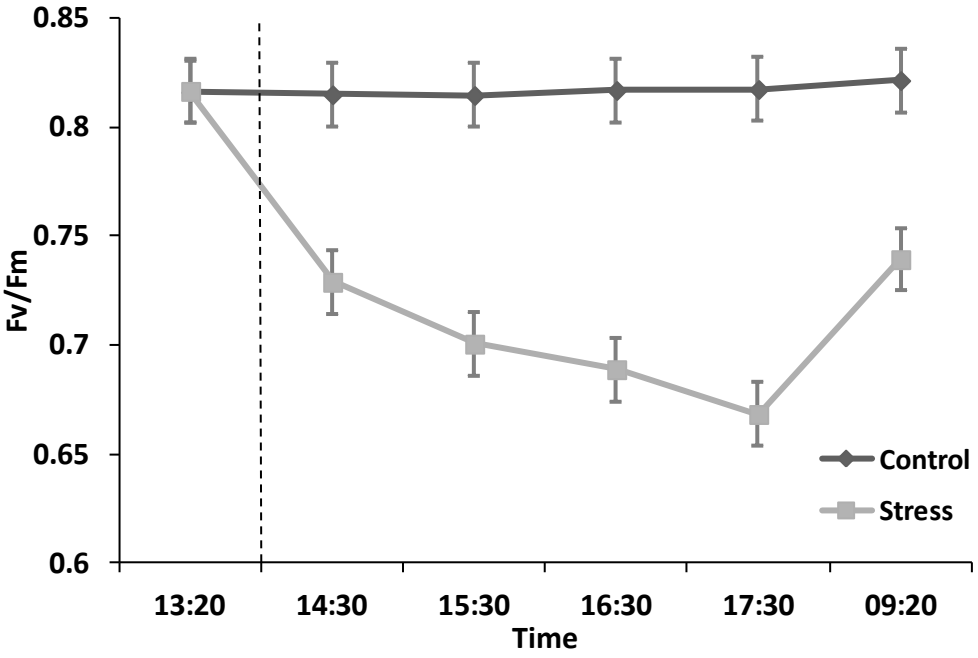

Fig. 9. Pooled chlorophyll fluorescence measured as Fv/Fm on garden rose clones 'Belinda's Dream', 'RADrazz', and 'Sir Thomas Lipton' using mature leaves on whole plants subjected to high temperature stress or control conditions in the greenhouse.

- Plants were moved to stress conditions at the dashed line. Stress conditions were imposed in a greenhouse with the shade opened and the pad and fan cooling shut off. Control conditions were in a greenhouse with temperature set points 25/20 ºC day/night.
- Measurement at 0920 $_{HR}$ was recorded after an overnight recovery period.
- Error bars represent one standard error of the mean.

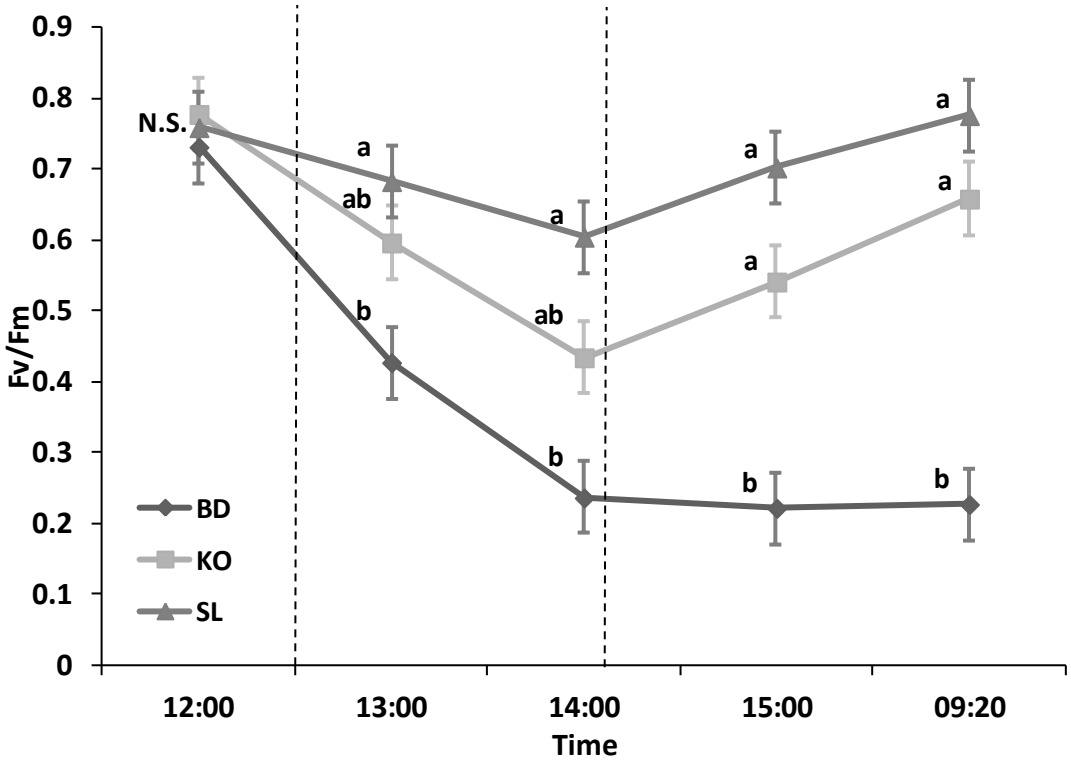

Fig. 10. Chlorophyll fluorescence measured as F_v/F_m using immature leaves of garden rose clones 'Belinda's Dream' (BD), 'RADrazz' (KO), and 'Sir Thomas Lipton' (SL) after whole plants were subjected to high temperature stress under greenhouse conditions.

- Error bars represent one standard error of the mean.
- Clones within time period not connected by the same letter are significantly different, $\alpha \leq 0.05$.
- First dashed line indicates start of stress conditions, second dashedl line indicate removal of plants from stress conditions. Stress conditions were imposed in a greenhouse with the shade opened and the pad and fan cooling shut off.
- Measurements at 0920 $_{HR}$ were recorded after an overnight period.

4.4.3 Experiment 3

No differences in CFL (0.813) were detected among J06A, VS, and SL prior to the heat chamber treatments, or after the three hour treatment (Fig. 11A). Five hour stress treatment resulted in a decrease in CFL with SL being the least affected and VS the most, where J06A was not different from either SL or VS (Fig. 11C). Both three and five hour stress treatments resulted in MTS showing differences among clones (Fig. 11B and D). No stress duration or measurement resulted in separation between J06A and SL. The MTS results for both the three and five h stress duration was more characteristic of results based on field performance of J06A and VS, as VS was less tolerant to heat stress than J06A in the field.

4.4.4 Experiment 4

CFL resulted in a difference between clones of both groups when leaves were subjected to high temperature stress in the water bath, but not when plants were stressed in the heat chamber (Table 12). MTS resulted in differences between clones of both groups when stress was applied in the water bath or heat chamber (Table 12). All differences detected between clones were in correspondence to field performance with the J06 lines having superior performance over SC and VS under high temperatures. A strong positive correlation ($r = 0.74^{***}$) was found between CFL and MTS across measurements taken on leaves stressed in the water bath. No correlation ($r = 0.21^{NS}$) was found between CFL and MTS measurements on leaves where plants were stressed in the heat chamber.

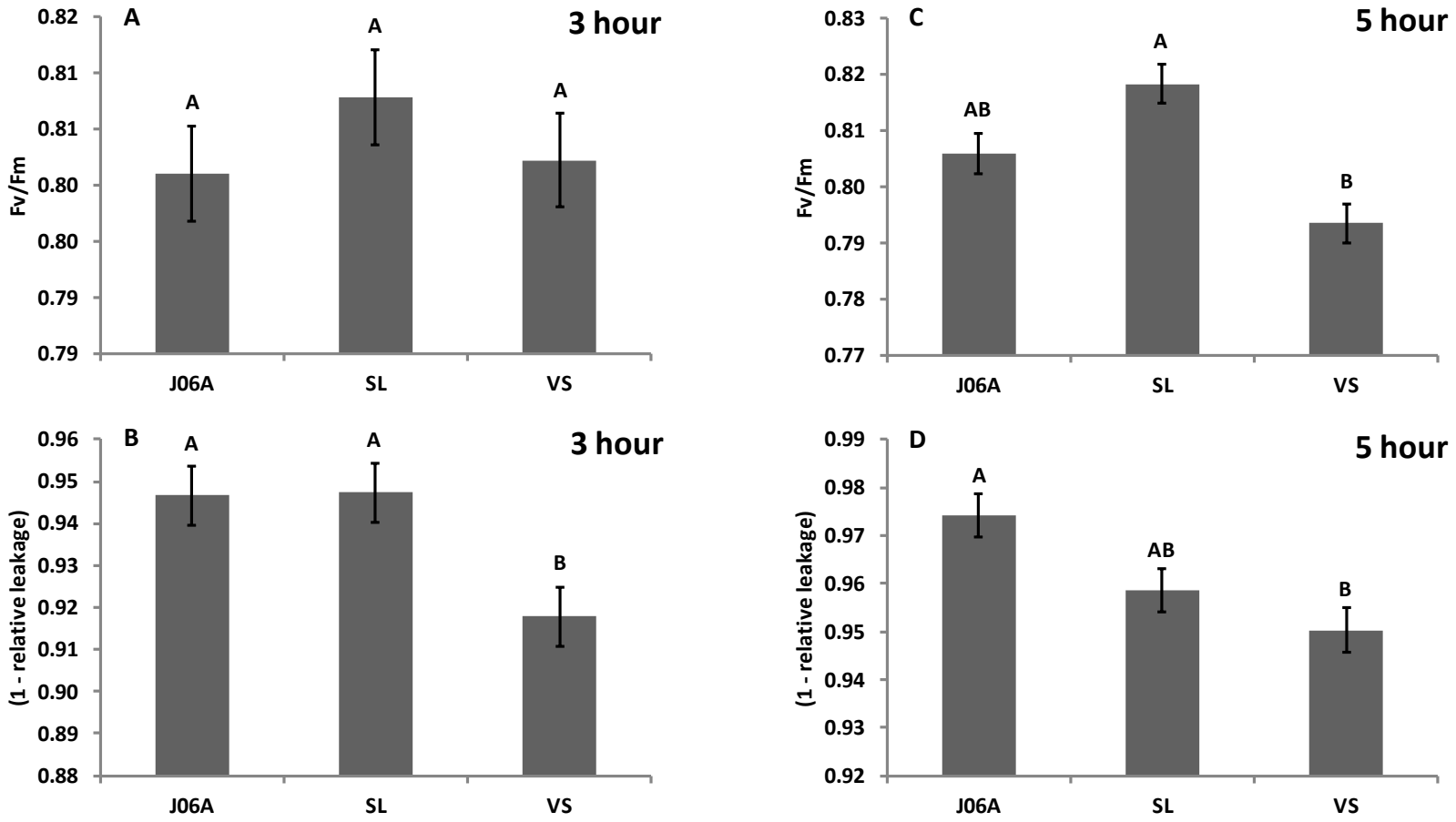

Fig. 11. Chlorophyll fluorescence measured as F_v/F_m (A, C) compared to membrane thermostability (MTS) measured as 1 - relative electrolyte leakage (B, D) on immature leaves of garden roses J06A, 'Sir Thomas Lipton' (SL), and 'Vineyard Song' (VS) subjected to high temperature stress in the heat chamber set at 42 ºC and 35 % RH for three (A, B) and five (C, D) hours.

- Error bars represent one standard error of the mean.
- Clones not connected by the same letter are significantly different, $\alpha \leq 0.05$ with Tukey's adjustment.

Table 12. Chlorophyll fluorescence (CFL) and membrane thermostability (MTS) measured on immature leaves stressed in a water bath (50 ºC for 45 min) and whole plants stressed in a heat chamber (44 ºC, 50 % RH for 3 hours).

		Group 1[z]		Group 2	
		J06B[y]	SC	J06A	VS
Water bath	CFL	0.443 ± 0.018 a[x]	0.371 ± 0.018 b	0.391 ± 0.052 a	0.210 ± 0.052 b
	MTS	0.906 ± 0.006 a	0.800 ± 0.006 b	0.626 ± 0.032 a	0.535 ± 0.032 b
Heat chamber	CFL	0.802 ± 0.006 a	0.798 ± 0.006 a	0.806 ± 0.010 a	0.771 ± 0.010 a
	MTS	0.980 ± 0.002 a	0.971 ± 0.002 b	0.975 ± 0.002 a	0.961 ± 0.002 b

- [z], Group 1 includes J06B and SC, Group 2 includes J06A and VS.
- [y], J06A and J06B: breeding lines, SC: 'Sweet Chariot', VS: 'Vineyard Song'.
- [x], Comparisons made between clones within groups. Clones not connected by the same letter are significantly different, $\alpha \leq 0.05$.

None of the plants showed any visible signs of stress immediately after removal from the heat chamber treatment. Evaluations ten days after the heat chamber treatment indicated that the heat chamber treatment was severe enough to cause visible signs of high temperature damage such as flower abscission (Fig. 6A and D), shoot burn (Fig. 6B), and leaf necrosis (Fig. 6C).

The total number of shoots with and without abscised flowers 10 days after the heat chamber treatment between J06B and SC resulted in a clone effect. 34 % (30/89) of J06B shoots had at least one abscised flower, whereas 55 % (29/53) of SC shoots had at least one abscised flower. After adjusting for the replicate effect, the odds of J06B not having abscised flowers was 1.63 times (95 % CI, 1.13 - 2.35) that of SC (Likelihood Ratio $\chi^2 = 6.99^{**}$).

SC was more susceptible to leaf necrosis than J06B, and resulted in 10 % (2/21) of the labeled J06B shoots and 29 % (6/21) of SC shoots showing signs of leaf necrosis. A significant clone effect was detected. The odds of J06B shoots not having leaf necrosis present was 2.84 times (95 % CI, 1.07 - 12.81) that of SC (Likelihood Ratio $\chi^2 = 4.41^{*}$).

Clone and treatment effects were significant for flower dry weight for heat chamber treated J06B and SC when compared to plants not exposed to the heat chamber. No interaction between clone and treatment was found. J06B has single to semi-double flowers whereas SC has double flowers. All flowers opened and were harvested between 14 and 25 days following the heat chamber treatment with the mean number of days to open flowers being 18 days. The mean flower dry weights for J06B and SC were 0.059

and 0.084 g respectively. Flowers from plants subjected to the heat chamber stress were 0.017 g lighter than flowers from plants not subjected to stress.

4.4.5 Experiment 5

Subjecting immature leaves from ten plants of J06B and VS to high temperature stress in the water bath resulted in a significant clone effect. In correspondence to results reported above, J06B leaked fewer electrolytes than VS showing greater membrane stability under high temperature stress (Fig. 12).

4.4.6 Power and sample size

The results from the power analysis predicts that using immature detached leaves stressed in a water bath would not be very effective in detecting MTS differences of 5 % between clones (Fig. 13A). To achieve a power of 0.8, 17 blocks (replicates) would be required to detect differences of 5 % between two clones, which might not be an efficient use of resources. The proposed protocol is likely to be effective in detecting MTS differences of 10 % and larger between clones (Fig. 13B and C). Using only 9 blocks would achieve a reasonable power (0.78) for detecting a 0.1 difference in MTS between 20 clones after adjusting for Tukey's multiple error testing (Fig. 13B).

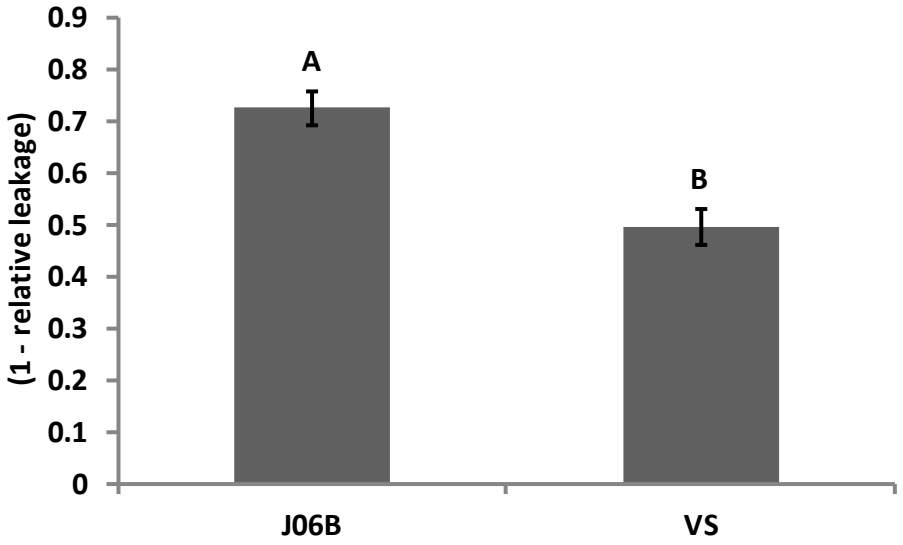

Fig. 12. Cell membrane thermostability (MTS) measured as 1 - relative electrolyte leakage on immature leaves of J06B and 'Vineyard Song' (VS) stressed in a water bath for 45 min set at 50 ºC.

- Error bars represent one standard error of the mean.
- Clones not connected by the same letter are significantly different, $\alpha \leq 0.05$.

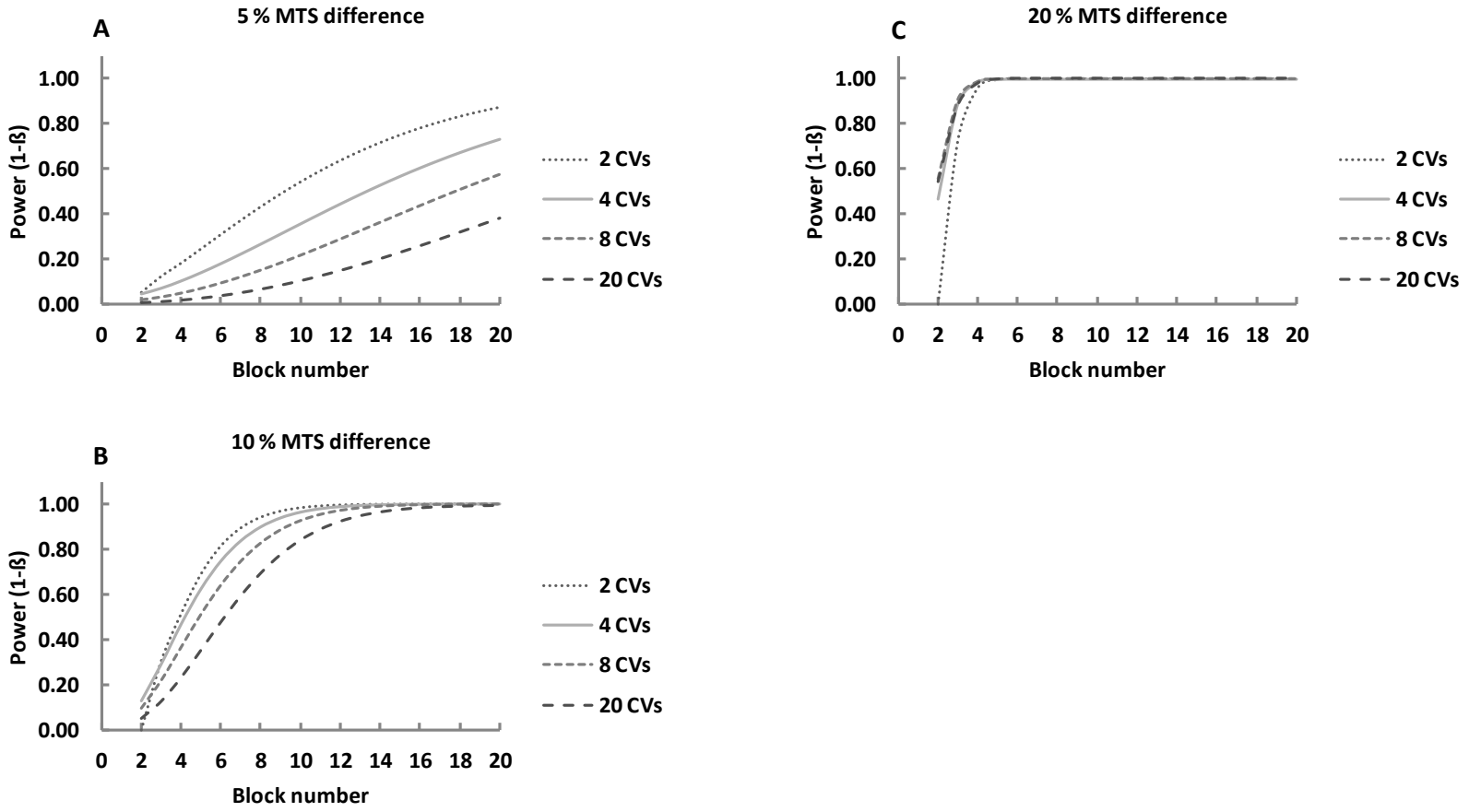

Fig. 13. Predicted power (1-ß) for detecting 5 (A), 10 (B), and 20 (C) percent difference in cell membrane thermostability (MTS) between differing number of clones screened in a randomized complete block design, when applying high temperature stress to immature leaves in a water bath set at 50 ºC for 45 min.

- α of 0.05, $\sigma_{residual}$ of 0.0438, and σ_{block} of 0.0873 were used in calculations.
- Tukey's honestly significant difference adjustment was applied to control for multiple testing error. Block number represents the number of complete blocks in experiment thus total number of plants = block number x number of cultivars.

4.5 Discussion

The effect of high temperature stress is dependent on temperature and duration. Optimizing the stress duration allowed for the maximization of the difference between BB and BD. Subjecting leaves to stress for 45 min at 50 ºC is similar with protocols used in other crops, although this experiment only optimized for stress duration and not temperature. The temperature where F_0 starts to increase sharply for *Rosa rugosa* is around 47 ºC, although Weng and Lai (2005) made use of a 20 min incubation period, and placed leaves directly in the water bath and not in plastic bags as in this experiment. A 1 h stress period at 50 ºC in a water bath was deemed effective for separating differences among 59 vegetable species based on electrolyte leakage (Kuo et al., 1993)

The summer of 2011 was extremely hot and dry in southeast Texas, with the average daily maximum temperature in College Station, TX for September being 39.9 ºC (Weather Underground Inc., 2012). This enabled the identification of material that performed both well and poorly under the hot conditions. Clones J06A and J06B were identified as tolerant and clones SC and VS were identified as susceptible.

The inability to detect differences among BD, KO, and SL based on detached leaves prompted whole plant stress assays. Although quite severe stress conditions were imposed on plants in the greenhouse, no difference among clones were detected when recording CFL on mature leaves. Recording CFL on immature leaves resulted in separation among clones. CFL recorded on mature leaves were successful in phenotyping an interspecific raspberry (*Rubus*) population for heat tolerance (Bravo,

2009) and when *Rosa rugosa* was included in a comparison of heat tolerance of 26 plant species (Weng and Lai, 2005), which is in contrast to the results presented.

Comparing CFL from immature leaves of BD, KO, and SL resulted in BD being the most susceptible and no differences observed between KO and SL. Immature leaves of BD were not able to recover to pre-stress F_v/F_m levels after an overnight period similar to high temperature susceptible chickpeas (*Cicer arietinum* L.) stressed at 50 ºC for four hours (Srinivasan et al., 1996). KO and SL did recover which was also reported for the more tolerant groundnuts (*Arachis hypogaea* L.) (Srinivasan et al., 1996).

SL performing as well as KO was unexpected due to KO having superior landscape performance compared to SL. KO was expected to have the smallest decrease in CFL followed by BD and then SL. Based on field performance under minimal input conditions in North-central Texas, KO performed better than both BD and SL in terms of flowering, overall landscape performance, and vigor. Whereas BD was more floriferous than SL, the two roses had comparable overall landscape and vigor ratings (Mackay et al., 2008). Vigor is highly influenced by black spot (*Diplocarpon rosae*) (Mackay et al., 2008) and the low vigor and overall ratings received by SL could, in part, be due to susceptibility to black spot (Helpmefind, 2012). Thicker leaves have been associated with high temperature tolerance in Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) (Kuo et al., 1988). SL is a *R. rugosa* hybrid displaying typical *R. rugosa* leaves which have been described as thick and leathery with pubescent abaxial and rugose adaxial surfaces (Bruun, 2005), all of which are traits associated with avoiding high temperature stress (Levitt, 1980). Flower production under high temperature stress might be

independent from the ability of a garden rose to maintain healthy foliage under such conditions.

Subjecting plants to stress in the heat chamber resulted in MTS being able to separate clones in accordance to field observations, whereas CFL failed. The inability of CFL to detect differences between clones subjected to stress in the heat chamber is in conflict to what was observed when stress was imposed under greenhouse conditions. Stress conditions in the greenhouse were more severe than those in the heat chamber. Temperatures in the greenhouse reached above 50 ºC whereas the heat chamber temperature was controlled at 42 ºC or 44 ºC. Plants subjected to stress in the greenhouse experienced an average PAR of 890 $\mu mol \cdot m^{-2} \cdot s^{-1}$, nearly 36 times that of the PAR in the heat chamber. Sharp increases in light intensity will decrease F_v/F_m (Björkman and Demmig, 1987), and the maximum PAR experienced under greenhouse stress conditions was 1531$\mu mol \cdot m^{-2} \cdot s^{-1}$. The differences in CFL detected among clones stressed in the greenhouse could be attributed to an additive effect of increased light intensity and higher temperatures than what was experienced in the heat chamber.

Both electrolyte leakage and chlorophyll fluorescence were successful in separating differences in high temperature tolerance among groundnut, soya bean (*Glycine max* L. Merr), chickpea, and pigeonpea (*Cajanus cajan* L. Millsp.) (Srinivasan et al., 1996). In contrast to the current results, Srinivasan et al. (1996) found CFL a useful indicator of high temperature tolerance when subjecting both detached leaves and whole plants to high temperature stress. Srinivasan et al. (1996) only recorded MTS from leaf disks stressed in a water bath, although Camejo et al. (2005) reported a

difference between two tomato lines measuring MTS on leaves when plants were stressed at 42 ºC for two hours. The reported correlation of 0.74 between CFL and MTS from leaves stressed in the water bath is in line with those reported by Srinivasan et al. (1996) (0.57 - 0.87), although different from their experiment here, CFL and MTS were recorded on the same leaves which allows for direct comparison between the two methods.

Subjecting garden roses BD and KO to two week sequential high temperature treatments (36/28 ºC day/night) resulted in elevated numbers of abscised flowers at the developmental stage two to three weeks before flowers opened, with BD being more susceptible to flower abscission than KO (Chapter II). Our results show that a short high temperature shock at a sensitive stage of development (flower bud 1 - 2 mm in diameter) can increase flower abscission in susceptible clones. Evaluating the presence and absence of leaf necrosis ten days after the heat chamber treatment indicated that SC was more sensitive to necrosis than J06B. The reduction in flower size was not different between J06A and SC. Flower abscission and leaf necrosis ten days after heat chamber treatment appear to be better indicators of high temperature susceptibility than flower size.

The result of J06B leaking less electrolytes than VS after subjecting ten replicates to high temperature stress in the water bath acts as proof of concept towards a difference in cell membrane thermostability between the J06 lines and, SC and VS. The power of a test depends on the smallest difference considered significant between treatments, α level, sample size, and the actual difference between treatments (Lenth,

2007). The power analysis predicts that differences in MTS greater than 10 % would consistently be detected between clones with fairly small replications, although the number of replications would have to be adjusted depending on the number of clones to be compared. Four replications would be sufficient to detect a 20 % difference comparing 20 clones whereas nine replicates would be sufficient to detect a 10 % differences when comparing 20 clones.

4.6 Conclusions

A series of consecutive experiments indicated MTS to be a more sensitive indicator of susceptibility to high temperature stress in garden roses than CFL. Evidence supports the use of immature leaves over mature leaves for use in phenotyping high temperature susceptibility. When plants were stressed at 44 ºC for three hours in the heat chamber, CFL was not drastically affected, only MTS was able to detect differences between clones. Subjecting immature leaves to stress in a water bath (50 ºC, 45 min) resulted in both CFL and MTS being able to separate differences between clones, although MTS was more consistent.

Overall landscape ratings do not necessarily reflect high temperature susceptibility. Landscape performance could be affected by any number of factors and some clones with poor landscape ratings can show adaptation for high temperature tolerance. It is unlikely that a laboratory test will ever completely replace field evaluation when selecting for adaptation to abiotic stress factors. Combining MTS from immature leaves stressed in a water bath with flower abscission and leaf necrosis after a high temperature shock in a heat chamber could be used in breeding programs to select

against the most susceptible plants, resulting in more efficient use of land for evaluating new material in the field.

CHAPTER V

FLOWER ABSCISSION AND LEAF NECROSIS ARE BETTER PREDICTORS OF LOW SUMMER FLOWER INTENSITY THAN ELECTROLYTE LEAKAGE IN 18 GARDEN ROSE (*ROSA ×HYBRIDA*) CLONES

5.1 Synopsis

Eighteen garden rose (*Rosa ×hybrida*) clones were evaluated for high temperature performance based on summer flower intensity (FI) over 2012 and 2013 in Mansfield, TX. Ten replicates of the same 18 clones were grown in the greenhouse. Cell membrane thermostability (MTS) was recorded after stressing leaves in a water bath (50 ºC, 45 min). Subsequently, plants with actively growing flowering shoots were subjected to a three hour stress in a heat chamber (44 ºC, 50 % RH). All the plants were evaluated for leaf necrosis and flower abscission ten days after the heat chamber treatment. Genotypic differences were found for all traits recorded. MTS did not correlate with summer FI. Leaf necrosis was negatively correlated to both MTS ($r = -0.59^{*}$) and summer FI ($r = -0.63^{**}$). Flower abscission was negatively correlated to summer FI ($r = -0.55^{*}$), and was most accurate in identifying clones with the worst summer performance. Results suggest that, subjecting actively growing plants to a heat chamber stress followed by recording flower abscission and leaf necrosis post stress be used in early selection against the most highly temperature susceptible plants. Such a system can aid

garden rose breeders to optimize their field space for selection of widely adapted material.

5.2 Introduction

According to Newton's law of motion, a force is always accompanied by a counterforce. The two forces are called the action and the reaction, and as a whole are known as a stress. A body subjected to stress is said to be in a state of strain. Stress such as described above is mechanical. Biological systems are constantly changing and a different definition of stress is required (Levitt, 1980). Larcher (2003) defines biological stress as "a significant deviation from the optimal conditions of life," whereas Taiz and Zeiger (2002) defines stress as "an external factor that exerts a disadvantageous influence on the plant." As Newton's law of motion works in on mechanical systems, so also do plants react on different levels (molecular, cellular, and whole plant) to counter the effect of stress inducing environments.

In crop plants, the most noticeable effect of high temperature stress is growth inhibition (Levitt, 1980), which can lead to major yield reduction (Wahid et al., 2007). High temperature stress is threatening crop production on a global level (Hall, 2001), and can be a limiting factor in subtropical climates like Texas (Wahid et al., 2007). Evaluating necrotic lesions on leaves after a high temperature stress is one of the earliest methods described in literature to assess the upper temperatures suitable for plant growth (Sachs, 1864), and has also been applied as a method for evaluating cold tolerance on thirteen garden rose cultivars (Hakam et al., 2000). Other methods used to evaluate high temperature stress includes cell membrane thermostability (MTS) measured by electric

conductivity based on the amount of electrolytes leaked from high temperature injured cell membranes (Hall, 1992).

Numerous crop plants are sensitive to high temperature stress during the early phases of reproductive growth. High day temperatures (35 ºC) are most severe in arresting inflorescence development in broccoli (*Brassica oleracea*) when stress is applied after initiation of the reproductive growth (Björkman and Pearson, 1998). High temperature stress during flower development can result in flower abscission of cowpeas (*Vigna unguiculata* L.) (Ahmed et al., 1992) and common beans (*Phaseolus vulgaris* L.) (Monterroso and Wien, 1990).

High temperature shock of garden roses during the visible bud stage of development led to increased levels of flower abscission (Chapter III and Chapter IV) and leaf necrosis on susceptible cultivars (Chapter IV). MTS recorded on leaves stressed in a water bath (50 ºC, 1 h) resulted in separation between susceptible and tolerant cultivars (Chapter IV).

High growing temperatures affect roses on various levels. High growing temperatures reduced the number of vegetative nodes on several cultivars of potted miniature roses (Grossi et al., 2004), but the effect on plant architecture is not uniform on garden roses (Chapter II). Increased growing temperatures resulted in decreased flower quality of greenhouse grown cut rose flowers by reducing size (Shin et al., 2001), color (Dela et al., 2003), and longevity (Marissen, 2001; Moe, 1975). Results from Chapter III indicated that a two week high temperature stress (36/28 ºC day/night) applied during the visible bud stage of development resulted in a significant reduction of

flower size in the garden roses 'Belinda's Dream' and 'RADrazz', whereas the natural logarithm of flower dry weight linearly decreased with increasing maximum daily temperatures two weeks before garden rose flowers opened (Chapter II).

Selecting for garden roses tolerant to high temperatures would contribute to the development of new cultivars adapted to a wide range of climatic conditions, one of the most likely limitations in rose sales (Byrne et al., 2010; Hutton, 2012). Roses are a perennial crop with long generation times, which can tie up breeding nursery space and resources for several years before a selection decision is made limiting breeding progress when compared to an annual plant. Any accurate method to select against poor performing plants prior to field establishment would be valuable to garden rose breeders. Here we conducted field evaluations on 18 garden rose cultivars for summer flower productivity and compared them with results of recently developed laboratory/greenhouse evaluation assays on the same cultivars. Suggestions on early selection against high temperature susceptibility are presented.

5.3 Materials and methods

5.3.1 Greenhouse and laboratory

Rooted cuttings of 18 *Rosa* L. accessions, from here on referred to as clones, were obtained from Seville Farms, Inc., Mansfield, TX, in January, 2013. The 18 clones included: 'Baby Betsy McCall' (BBM), 'Baby Darling' (BDL), 'Born Free' (BF), 'Carmela' (CA), 'Cal Poly', (CL), 'Fair Molly' (FM), 'Galaxy' (GA), J06-30-3-3

(J06A), ‘Julie Link’ (JL), Moore ND (ND), ‘Ring of Fire’ (RF), ‘Ruby Magic’ (RM), S3-19, S3-31, ‘Sunday Brunch’ (SB), and ‘Sweet Chariot’ (SC).

The cuttings were planted in 3.7 L plastic pots in a bark-based, soilless media (Fafard 52 Mix, Conard Fafard Inc., Agawam, MA). The plants were grown in the horticulture greenhouse in College Station on Texas A&M University’s west campus, and kept at temperature set points 25/20 ºC day/night. The temperature in the greenhouse was controlled by natural gas heaters and a pad and fan cooling system. All pests were controlled as necessary and plants were irrigated and fertilized with each irrigation as required with a 200 $mg \cdot L^{-1}$ 15N – 5.4P – 14.1 K liquid fertilizer, except for weekends when plants were irrigated with water only.

Once well established, ten (replicates) plants of each of the 18 clones were selected based on uniformity of size and shape. Ten greenhouse benches served as separate blocks where a complete set of clones was housed for the remainder of the experiment. The clones were randomized on each bench. Plants were pruned to encourage new growth. Pruning commenced in the last week of February with block one, followed by pruning the second block three days later. Three days later the next block was pruned until pruning of block ten. This was done to synchronize growth within a block and separate stages of growth between blocks.

When at least two actively growing flowering shoots on each plant per block had flower buds between 1 - 2 mm in diameter the block was ready to be screened. Cell membrane thermostability (MTS) measurements based on a conductivity test was recorded on two immature leaves (subsamples) directly above the last unfolded leaf on

an actively growing shoot on each plant. The MTS protocol was performed as described in Chapter IV (4.3.2). The stress conditions were maintained at 50 ºC for 45 min.

On the day following the start of the MTS procedure, plants from the same block were subjected to high temperature stress in the heat chamber, as described as in Chapter IV (4.3.3). The heat chamber was set at 44 ºC and 50 % RH, and plants were stressed for 3 hours. Stress treatments started at 0800 $_{HR}$. Two actively growing shoots with flower buds 1 - 2 mm in diameter were labeled before the stress treatment. Immediately following the heat chamber treatment, plants were transferred back to the greenhouse.

All plants were evaluated for leaf necrosis and flower abscission ten days post heat chamber treatment. Leaf necrosis was scored on the most affected leaf on each of the two labeled shoots per plant. Necrosis was scored on a 1 - 10 scale starting with 1 being 0 to 5 % necrosis present on the leaf surface, and then increasing in 10 % increments to 10 where 95 to 100 % of the leaf surface area was necrotic. Flower abscission was scored on a binomial scale for each of the two labeled shoots per plant. Flower abscission was scored as a 1 if the terminal flower bud abscised.

5.3.2 Field evaluation

A large collection of germplasm from the Texas A&M Rose Breeding Program was planted in the field at Seville Farms, Inc., Mansfield, TX in February, 2010. All plants were randomly planted and have 1 - 2 replicates per plant. The plants received irrigation and pest management as needed. All the plants were pruned in February of each year. The 18 clones used in this experiment are contained within this collection. Field evaluation for summer productivity took place in August of 2011 and 2012. High

temperature performance was based on flower intensity, and was scored on a 0 - 9 scale based on the percentage of the plant surface covered with flowers such that, 0 = no flowers, and 9 = more than 90 % plant surface, with 10 % increments between 0 and 9.

5.3.3 Statistical analysis

All statistical analyses were performed using JMP (version 9.0; SAS Institute Inc., Cary NC 1989 - 2010). The data from the greenhouse and laboratory experiments were analyzed as randomized complete block layouts with subsampling by fitting a mixed least squares model with replicates as random. Differences between clone means were tested using Tukey's Honestly Significant Difference test. A natural logarithm transformation was applied to leaf necrosis scores to increase model fit. Nominal logistic regression was used to analyze flower abscission results and predicted probability of flower abortion was computed from the log of the odds for flower abortion. Summer FI was averaged per clone for each year, and over both years. Pearson's and Spearman's rank correlation coefficient between field evaluations and greenhouse and laboratory results were computed.

5.4 Results

MTS resulted in significant differences among clones (Table 13). The smallest significant differences between two clones were 0.144 after adjusting with Tukey's Honestly Significant Difference. MTS divided clones into five overlapping grouping with J06A (0.738) having the highest stability and ND the greatest cell membrane damage (0.343) (Table 13).

Evaluating leaf necrosis on the most affected leaf on each of pre-labeled actively growing shoots resulted in significant differences among clones (Table 13). The data is separated into four overlapping groupings with BDL having the greatest amount of leaf necrosis and FM with the least (Table 13).

A significant clone effect for flower abscission was detected. There were four clones (BDL, CH, RF, and S3-31) that had more than 70% flower abscission and two (JL, SM) with less than 20% abscission (Fig. 14).

The average daily maximum and minimum temperatures in Mansfield for July and August were 36.4 and 23.6 ºC for 2012 and 34.6 and 23.9 for 2013 respectively, and the average relative humidity was 61% for both years (Weather Underground, Inc., weather station KTXMANSF11). The average summer FI among the 18 clones ranged between 0.5 and 5.5, with clones CA, GA, and J06A averaging 5.5 and BDL averaging 0.5 (Table 13). No correlation between MTS and summer FI was found. A significant negative correlation ($r = -0.57^{*}$) was found between MTS and leaf necrosis (Table 14). A significant correlation between leaf necrosis and summer FI ($r = -0.63^{**}$) were detected. Flower abscission was significantly negatively correlated with summer FI ($r = -0.55^{*}$). A significant positive correlation between flower abscission and leaf necrosis ($r = 0.59^{**}$) was detected. Both Pearson's and Spearman's rank correlation coefficients gave similar results (Table 14).

Table 13. Cell membrane thermostability (MTS) after water bath treatment (50 ºC for 45 min), leaf necrosis (± 1 standard error) tend days post high temperature stress in the heat chamber (44 ºC, 50 % RH, for 3 h), and average summer flower intensity (FI) over 2012 and 2013.

Clone	MTS	Leaf Necrosis	Summer FI
Baby Betsy McCall (BBM)	0.416 ± 0.03 de[z]	2.555 ± 1.168 bcd	4.50
Baby Darling (BDL)	0.403 ± 0.43 de	8.492 ± 1.089 a	0.50
Born Free (BF)	0.462 ± 0.06 cde	2.967 ± 1.218 bc	4.50
Carmela (CA)	0.632 ± 0.049 ab	2.572 ± 1.22 bcd	5.50
Chelsea (CH)	0.491 ± 0.051 bcd	4.142 ± 1.242 b	3.50
Cal Poly (CL)	0.436 ± 0.049 cde	4.498 ± 1.242 ab	4.50
Fair Molly (FM)	0.533 ± 0.05 bcd	1.463 ± 1.12 d	4.75
Galaxy (GA)	0.487 ± 0.48 bcd	2.821 ± 1.138 bcd	5.50
J06-30-3-3 (J06A)	0.738 ± 0.029 a	1.934 ± 1.194 cd	5.50
Julie Link (JL)	0.515 ± 0.046 bcd	3.712 ± 1.205 bc	4.50
Moore ND (ND)	0.343 ± 0.037 e	3.606 ± 1.222 bc	3.00
Ring of Fire (RF)	0.394 ± 0.042 de	4.64 ± 1.207 ab	3.50
Ruby Magic (RM)	0.44 ± 0.055 cde	4.462 ± 1.207 ab	4.25
S3-19	0.402 ± 0.048 de	4.899 ± 1.175 ab	5.00
S3-31	0.518 ± 0.043 bcd	4.172 ± 1.146 b	3.50
Sunday Brunch (SB)	0.4 ± 0.052 de	4.021 ± 1.108 b	4.50
Sweet Chariot (SC)	0.571 ± 0.036 bc	3.516 ± 1.185 bc	2.00
Saint Mary (SM)	0.523 ± 0.064 bcd	2.632 ± 1.19 bcd	4.00

- [z], Clones not connected by the same letter are significantly different, $\alpha \leq 0.05$ with Tukey's adjustment.

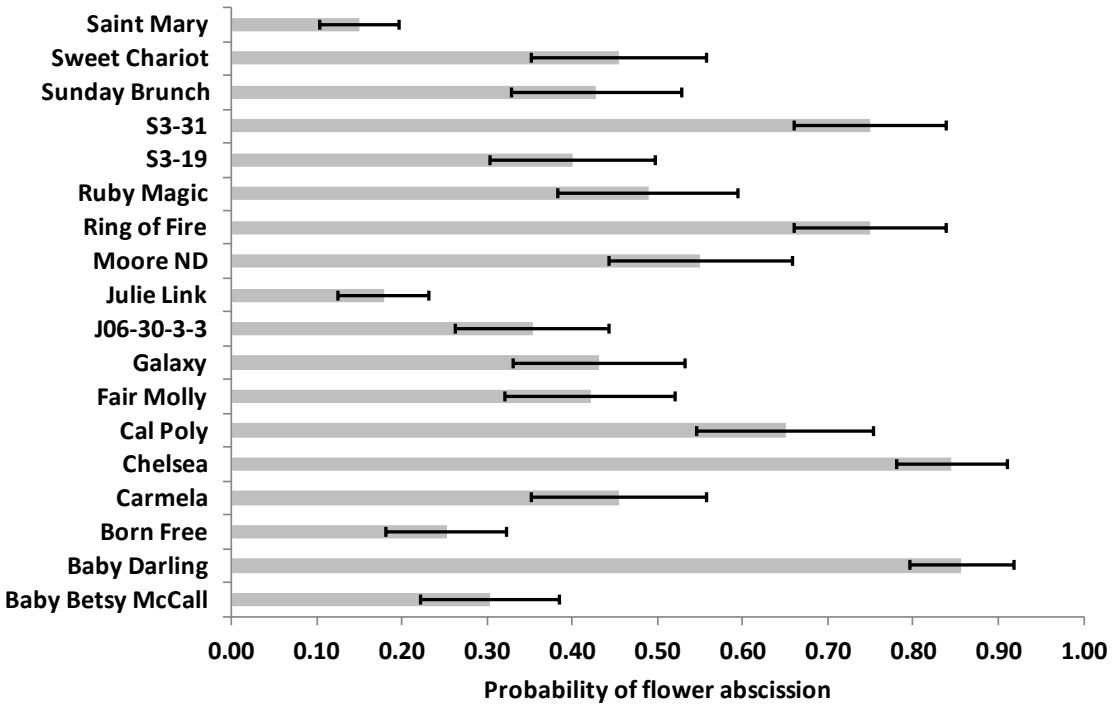

Fig. 14. Probability of flower abscission ten days after a 3 h stress in the heat chamber (44 ºC, 50 % RH).

- Error bars represent the 95 % confidence interval.

Table 14. Field evaluation of summer flower intensity (FI) correlated with cell membrane thermostability (MTS), leaf necrosis, and the probability of flower abscission (P Abscise) measured on 18 garden rose clones.

	Pearson's[z]			Spearman's rank[z]		
	MTS	Leaf necrosis	P abscise	MTS	Leaf necrosis	P abscise
FI summer'12[y]	0.39 NS	-0.60 **	-0.601 **	0.31 NS	-0.42 NS	-0.58 *
FI summer'13	0.27 NS	-0.58 *	-0.431 NS	0.38 NS	-0.53 *	-0.42 NS
Average FI summer	0.35 NS	-0.63 **	-0.545 *	0.35 NS	-0.50 *	-0.54 *
MTS	-	-0.57*	-0.274NS	-	-0.64 **	-0.30 NS
Leaf necrosis	-	-	0.591**	-	-	0.60 **

- [z], Pearson's correlation coefficient and Spearman's rank correlation coefficient.
- [y], Summer FI rating for 2012 and 2013, summer FI averaged over 2012 and 2013.
- [NS], [*], [**], Nonsignificant and significant at P value 0.05 and ≤ 0.01.

5.5 Discussion

MTS revealed differences among clones. In the establishment of the MTS protocol (Chapter IV), J06A had lower electrolyte leakage than the more susceptible SC, which again was the case in this experiment. Judging high temperature performance based on summer FI, J06A performed superior to SC with an average summer FI of 5.5 compared to 2.0 of SC. However, when looking at the Pearson's or Spearman's rank correlation including the 16 other clones, MTS was not correlated to summer FI which was opposite to what was expected.

If selection against susceptibility to high temperature stress was based purely on MTS one might select against the clones with the greatest amount of electrolyte leakage such as those with de and e grouping based on mean separation tests. These clones would include: BBM, BDL, S3-19, SB, RF, and ND. A summer FI below 4 is not acceptable for commercial performance and selection based purely on MTS would have discarded BBM, S3-19, and SB with mean summer FI greater than 4, thus discarding material of commercial summer FI potential.

MTS was significantly correlated with leaf necrosis ($r = -0.57^{*}$). Direct injury to the cell membranes due to high temperature injury can be estimated by electrolyte leakage, while indirect injury due to high temperature stress can be expressed as necrotic lesions on the leaves (Levitt, 1980). Bilger et al. (1984), used leaf necrosis scores during the development of chlorophyll fluorescence assays to evaluate high temperature tolerance in plants. A strong positive correlation ($r = 0.74^{***}$) was reported between MTS

and chlorophyll fluorescence in Chapter IV. It was expected that MTS would be correlated with leaf necrosis.

In Chapter IV, SC had a greater probability to show leaf necrosis after treatment in the heat chamber than J06A. Results from this experiment did not show a significant difference in leaf necrosis between J06A and SC, even though SC had a larger (3.5) mean leaf necrosis than J06A (1.9). This indicates that the variation in leaf necrosis response was large and that either a greater sample size is required to better separate differences between clones like J06A and SC, or further optimization of stress and temperature duration to evaluate leaf necrosis is needed.

During the development of the MTS protocol (Chapter IV), only a small number of clones (2 - 4) with differences in high temperature susceptibility were compared to each other. Similarly Camejo et al. (2005) only compared two tomato cultivars in their work. The cultivars used by Camejo et al. (2005) and in Chapter IV were both different in high temperature performance and cell membrane thermostability. MTS was a good indicator of high temperature performance on four species of food legume with differences among and within the species (Srinivasan et al., 1996). There are reports on both the presence and absence of correlations between genotypic differences and cell membrane thermostability based on leaf disks assays (Hall, 1992). Results presented here provide evidence that the use of MTS is not a reliable indicator of high temperature susceptibility in garden roses.

Leaf necrosis was significantly inversely correlated ($r = -0.63^{**}$) with summer FI. A large number of the clones were not statistically different from each other based on

leaf necrosis. BDL performed the worst. BDL had a mean spring FI of 5 (data not presented), and clearly under performs in the summer, and BDL's low summer FI is likely not due to not being a floriferous clone. If selecting based on leaf necrosis scores one would be able to accurately select against seedlings performing similarly to BDL. Even though the selection against BDL would be accurate, not enough material would be discarded based on leaf necrosis alone. Optimized experimental conditions and refinement of how leaf necrosis is evaluated may result in better resolution to detect differences between clones.

Differences in the probability of flower abscission were present among the 18 clones used in this experiment. Tolerance to flower abscission is an important trait in food crops such as beans (Monterroso and Wien, 1990), broccoli (Björkman and Pearson, 1998), and cowpeas (Ahmed et al., 1992) where the arrest of inflorescence development (broccoli), and flower and pod abscission (beans and cowpeas) significantly reduces yield and quality of the products. It is expected that new garden rose cultivars perpetually produce a large number of flowers. Flower abscission reduces the "yield" and quality of the plant's performance during the hotter times of the year. The four clones with the highest probability of flower abscission were BDL, CH, RF, and S3-31, all of which had an average summer FI of 3.5 except for BDL which averaged 0.5. Based solely on flower abscission one could accurately select against the majority of the worst performers in terms of summer flower productivity.

BDL performed significantly worse than the other clones and could be classified as an outlier. Removing BDL from the analysis changed the correlations of summer FI

with leaf necrosis (r = -0.40, P value = 0.11) and flower abscission (r = -0.46, P value = 0.06). The negative correlation between MTS and leaf necrosis (r = -0.58, P value = 0.02) remained significant. The change in the correlation between flower abscission and summer FI to be only marginally significant is probably due to the small sample size. With only 18 data points outlier values could significantly impact the result of a test. The fact BDL ranked very poorly compared to the other clones used in this experiment is not a sound basis for omitting it from the analysis.

5.6 Conclusions

Genotypic differences among the 18 clones were found for all traits evaluated, although not all traits correlated with summer flower productivity. The two clones (J06A and CA) with least amount of electrolyte leakage had good summer flower productivity. The evidence from this experiment suggests that membrane thermostability alone may not be a large enough factor in high temperature performance to be a reliable method for selection against the worst performing plants prior to costly establishment for field evaluation.

Results presented here suggest that electrolyte leakage may not be as effective in early selection against high temperature susceptibility in garden roses as reported in Chapter IV. High probability of flower abscission and increased leaf necrosis after a high temperature treatment in a heat chamber appears to be effective in selecting against high temperature susceptibility. In doing such evaluations it would be wise to include a high temperature susceptible and tolerant clone as points of reference. Depending on the number of replicates required, evaluating leaf necrosis and flower abscission were low

cost, easier, and less labor intensive than taking MTS measurements. Breeders focusing on adaptation to high temperatures could employ these methods to enable their programs to better use valuable breeding nursery space such that greater numbers of better adapted material could be screened in the field leading to the release of more widely adapted garden rose cultivars.

CHAPTER VI

CONCLUSION

The work described in this dissertation has first focused on quantifying and describing variation in response to high temperature stress of garden roses under field conditions. Secondly the work herein is focused on developing an early screening protocol to enable breeders of garden roses to select against the worst performing material prior to field establishment.

Yield reduction due to high temperature stress is commonly quantified by the yield produced by a specific crop on a per area basis. Yield of garden roses can be quantified as landscape performance, and was rated on a 1 - 5 scale at the Texas A&M Rose Breeding Program. Landscape performance is influenced by the ability of a rose to maintain healthy foliage cover and to have a high percentage of the plant perpetually covered in blooms. In terms of landscape performance under high temperatures the work presented in this dissertation focused mostly on summer flower productivity and dry weight of flowers.

Chapter II provided evidence that the average daily maximum temperatures for days 8 - 14 (2WkMaxºC) before a flower opens significantly affected the dry weight of the flower in conditions with a relatively high year round relative humidity (61.7 %). Evidence towards genotypic differences among garden roses with regards to reduction in flower size with increasing growing temperatures is presented. Fourteen adapted garden

rose cultivars were divided into two groups with a 4.28 and 6.45 % reduction in size for every 1 ºC increase in 2WkMaxºC.

Rose flowers were sensitive to two week high temperature treatments around the visible bud stage of development (Chapter III), which resulted in increased flower abscission in the sensitive cultivar and roughly a 42 % reduction in flower size for both cultivars (‘Belinda’s Dream’ and ‘RADrazz’). Flower buds (1 - 2 mm in diameter) subjected to high temperature shock treatments (44 º for 3 h) were sensitive to abscission and revealed genotypic differences. Cultivars with higher summer flower intensity had a lower propensity towards abscission than those with lower summer flower productivity (Chapters IV and V).

After a number of experiments investigating the use of chlorophyll fluorescence and cell membrane stability as predictors of susceptibility to high temperatures, initial results favored cell membrane stability over the use of chlorophyll fluorescence (Chapter IV). Subjecting a wider range of genotypes to high temperature stress provided evidence that cell membrane thermostability alone had no correlation to summer flower productivity (Chapter V). Although cell membrane thermostability under high temperatures likely plays some role in productivity under high temperatures, the trait is probably far too complex to be predicted by a single test such as membrane electrolyte leakage.

Numerous factors such as adaptation to avoid and or endure high temperature stress are predicted to play a part in what is observed as summer flower productivity by the breeder. At this point no rapid phenotyping protocol is presented. Current evidence

suggests that no rapid test in the laboratory or greenhouse will allow for prediction of visual response in the field to high temperature conditions.

Although no method for selecting the best material is presented, the results presented in Chapter V suggest that subjecting plants to a three hour high temperature (44 ºC, 50 % RH) shock in a heat chamber when flower buds are sensitive (1 - 2 mm diameter) would allow the breeder to discard the most inferior material based on propensity towards flower abscission and the severity of leaf necrosis ten days after stress. Genetic gain from selection in crops with long generation time is constrained by the time it takes for a selection cycle. Early selection against the worst performing material will help breeders increase gain from selection per year and better utilize their field space and labor.

The rose is a complex crop, as is portrayed by *Rosa* taxonomy. Linnaeus noted in his Species plantarum (p. 492, translated) "The species of the genus *Rosa* are difficult to distinguish and determine, I have the impression that nature combines just for fun a number them and then forms a new one out of the lot, those who have seen only some distinguish them more easy than those which have examined a lot" (Linnaeus, 1753). So might initial results from examining the differences in high temperature susceptibility among a few roses have led to the impression that phenotyping for high temperature susceptibility in roses would be simple. Further examination proved the task more daunting.

A complicating factor in high temperature field performance could be that landscape performance is subjective and that a more objective measurement of high

temperature field performance is required. Summer flower intensity combined with experiments separating out the factors involved in flower size reduction such as: petal number and petal size may prove useful in quantifying high temperature performance in roses.

A series of experiments from the early 1980's reported on the feasibility of selecting towards low energy input (reduced temperature and light intensity) cut roses. The goal was to reduce the cost involved in greenhouse production of cut roses in Europe. The result was that sufficient genetic variation was present and genetic gain from selection was predicted (De Vries et al., 1980; De Vries et al., 1982). History showed that the majority of cut rose production moved from Europe to South America and Africa, and the progress towards selecting for low input cut roses is unknown.

The long term goal of this project was to develop garden rose cultivars adapted to high temperature conditions present in large areas of the southern U.S. (Chapter I). Evidence towards ample genetic variation for performance under high temperature conditions was presented in this dissertation. The success of the long term goal to produce garden roses adapted to ever increasing temperatures will depend on continued research and further field selection in combination with early screening protocols.

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